UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: 4/11/2013

SUBJECT: Demiditraz: Human Health Risk Assessment to Support Section 3 New Use for Proposed Dog Spot-On Use

PC Code: 577501	DP Barcode: D410552
MRID No.: Not Applicable	Registration No.: 1007-OT
Petition No.: Not Applicable	Regulatory Action: Section 3 Registration
Assessment Type: Single Chemical, Non-Aggregate	Reregistration Case No.: Not Applicable
TXR No.: Not Applicable	CAS No.: 944263-65-4

FROM: Wade Britton, MPH, Risk Assessor and ORE
Linda Taylor, Ph.D., Toxicologist
Risk Assessment Branch V/VII
Health Effects Division (HED; 7509P)

THROUGH: William H. Donovan, Ph.D., Chemist
And
Michael Metzger, Branch Chief
Risk Assessment Branch V/ VII
Health Effects Division (7509P)

TO: Bewanda Alexander, Risk Manager
Richard Gebken, PM 10
Insecticide Branch
Registration Division (RD; 7505P)

Table of Contents

1.0 Executive Summary

Demiditraz, 2-[(1S)-1-(2,3-Dimethylphenyl)ethyl]-1*H*-imidazole is an acaricide which acts on the octopamine nervous system in invertebrates and acts on neurotransmitter systems, such as the alpha 2 adrenergic receptor in mammals. Demiditraz is proposed by the submitter, Pfizer Animal Health, as a spot-on liquid product for dogs to control ticks and mites. There are currently no active registrations for demiditraz. A human health risk assessment was previously conducted for the proposed dog spot-on product[1] which identified risks of concern for the use. This document updates the prior assessment with incorporation of additional exposure and toxicity data submitted since the prior assessment, and uses the 2012 Health Effects Division (HED) Residential Standard Operating Procedures (SOPs) to assess residential exposure and risk.

Proposed Use Profile: The proposed dog spot-on product, 1007-OT, contains two active ingredients; demiditraz (14%) and fipronil (4.8%). This human health risk assessment only addresses human health risk from potential exposures to demiditraz. The proposed product is labeled for application to four dog weight ranges: small, 11 to 20 pounds (lbs); medium, 21 to 33 lbs; intermediate, 34 to 50 lbs; and large, 51 to 66 lbs.

Exposure Profile: Non-occupational (residential) use of the dog spot-on product is anticipated to result in dermal exposures for adult handlers. In addition, residential post-application dermal exposures are expected for adults and children, and incidental oral exposures for children. Occupational dermal exposures are expected from use of the proposed spot-on product by veterinarians, veterinary assistants, and groomers. Inhalation exposures are not anticipated from either occupational or residential use of the spot-on product.

Current HED policy requires assessment for occupational handler and residential post-application exposures of short- (1 to 30 days), intermediate- (1 to 6 months), and long-term (greater than 6 months) exposures from pet spot-on products due to the preventative nature of pet products and the potential for extended usage in more temperate parts of the country. Residential handler exposure is assumed to be short-term due to the intermittent nature of homeowner spot-on applications (i.e., once monthly treatment).

Toxicity/Hazard: The nervous system is the primary target organ for demiditraz. Pfizer Animal Health maintains that demiditraz affects neurotransmitter systems such as the alpha-2 (α2) adrenergic receptor in mammals. There are no chronic studies for demiditraz; however, the toxicological database is adequate for selecting endpoints for a human health risk assessment and is considered complete because all the data requirements for a non-food use have been satisfied.

A database uncertainty factor (UF_{DB}) of 10X was retained in the previous human health risk assessment of the proposed demiditraz dog spot-on product based on the lack of developmental neurotoxicity (DNT) and non-rodent (dog) studies. The data requirement has been satisfied with submission of rat developmental neurotoxicity and dog subchronic oral toxicity studies, and the dog was not found to be more sensitive than the rat to the neurotoxic effects of demiditraz.

[1] W. Britton. Demiditraz: Human Health Risk Assessment for Proposed Dog Spot-On Use. D378783. 11/30/2010.

Accordingly, the additional UF_{DB} has been removed due to satisfaction of the required study requirements.

Demiditraz has not been classified for carcinogenic potential. Rat and mouse carcinogenicity studies with demiditraz have not been submitted, but are not required for the proposed non-food/non-feed use. All studies conducted for mutagenicity/genetic toxicity were determined to be negative and, therefore, there is no concern for mutagenicity/genetic toxicity for demiditraz. There are also no reproductive toxicity concerns. No specific immunotoxicity was identified in a guideline immunotoxicity study.

The database for demiditraz is complete, which includes a developmental neurotoxicity assessment; and there is no evidence of increased susceptibility and no residual uncertainties with regard to pre- and/or postnatal toxicity following *in utero* exposure to rats or rabbits and pre- and/or postnatal exposures to rats.

An incidental oral endpoint for risk assessment of all exposure durations was selected from the oral subchronic neurotoxicity study in the rat with a no observed adverse effect level (NOAEL) of 5 mg/kg/day. At the study lowest observed adverse effect level (LOAEL) of 25 mg/kg/day, clinical signs of neurotoxicity, decreased motor activity, and decreased body weights were observed in both sexes. The total uncertainty factor applied for assessment of incidental oral exposures and risks is 100X.

Dermal endpoints for all durations of risk assessment were selected from the route-specific subchronic dermal toxicity study in the rat with a LOAEL of 100 mg/kg/day. A NOAEL was not identified. Alterations in motor activity and grooming behavior were observed at all dose levels. An additional uncertainty factor, UF_{LOAEL}, of 3X has been retained due to the lack of a NOAEL (i.e., LOAEL to NOAEL extrapolation). The total uncertainty factor applied for assessment of dermal exposures and risks is 300X.

Residential Exposure and Risk Estimates: There is a potential for residential exposures from the use of the proposed demiditraz spot-on for dogs. Short-term residential handler dermal exposure and risk was assessed and is not of concern (i.e., MOES are > 300). Inhalation exposures for residential handler and from post-application exposures to the proposed spot-on product are assumed to be negligible.

Residential post-application dermal and incidental oral exposures (all durations) were combined for all children 1 to < 2 years old exposure scenarios assessed. Children's combined exposures were presented using the aggregate risk index (ARI) approach. This approach was required to assess combined post-application exposure and risks for children 1 to < 2 years old because the LOCs are not the same for dermal and oral routes of exposure (i.e., dermal, 300; and incidental oral, 100). For adults, only post-application dermal exposure is anticipated from contact with a demiditraz treated dog.

Pfizer Animal Health submitted a pet residue transfer study in support of the proposed demiditraz spot-on use. These data were used in conjunction HED's 2012 Residential SOPs to refine the assessment of residential post-application exposures to demiditraz. Day of application

(Day 0) residues were used to assess all durations of residential post-application exposure. For the purpose of characterizing longer-term exposures and risks (i.e., intermediate and long-term), multi-day exposure risk estimates were also quantified by use of the average of percent residue using transfer values predicted from Days 0 to 30 (i.e., the proposed product re-treatment interval).

Residential post-application adult dermal, and combined children 1 to < 2 years old exposures are not of concern for all durations of exposure (i.e., adult dermal MOEs are > 300; and children 1 to < 2 years old ARIs are > 1) with use of Day 0 residue data. Exposures estimated for longer-term exposures using 30 day average residue data are approximately 7X below (MOEs 7X greater) those estimated for all durations using Day 0 data.

Occupational exposures to veterinarians, veterinary assistants, and groomers may occur from the application of the proposed spot-on product to dogs. Dermal exposure assessments (all durations) were conducted to assess occupational handlers applying the demiditraz spot-on to dogs. Occupational handlers of the proposed product could potentially treat up to 170 dogs per day without resulting in risks of concern for all durations assessed (i.e., MOEs are ≥ 300). Occupational handler inhalation exposure is expected to be negligible and was not quantitatively assessed. Further, a quantitative assessment of occupational post-application exposure from the proposed demiditraz spot-on product was not conducted. Occupational post-application activities are expected to be significantly less than residential post-application exposures. That is, dogs are expected to be treated and returned to their owners such that post-application contact will be negligible.

Residential Aggregate Exposure and Risk Estimates: An aggregate exposure assessment, which combines exposures from different sources and routes, is typically conducted for non-food/ non-feed chemicals when there is potential for human exposure through water and residential pathways. Demiditraz currently has no registered food/feed uses and no drinking water residues are expected to result from the proposed pet use; therefore, aggregate exposure includes only the exposure from the dog spot-on use.

Environmental Justice: Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment. Only non-dietary exposures were considered.

2.0 HED Recommendations

HED has evaluated the exposure and toxicology databases, and assessed occupational and residential exposure and risk from the proposed demiditraz dog spot-on use and no issues have been identified which would preclude granting the Section 3 registration of the proposed use.

2.1 Data Deficiencies

There are no exposure or toxicology data deficiencies to prohibit product registration.

2.2 Tolerance Considerations

The dog spot-on product is the only use proposed for demiditraz and is classified (a non-food/non-feed use); therefore, there are no tolerances to evaluate.

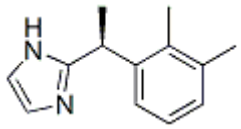
2.3 Label Recommendations from Occupational and Residential Assessment

As currently proposed, 1007-OT labeling does not include directions regarding the manner in which the spot-on should be applied to dogs. Label directions should include the following: how the application tube is prepared for treatment (i.e., snap or twist open); whether preparation of the application site is required (e.g., parting dog's hair coat); and where on the dog's body the product should be applied (i.e., single or multiple application points). Such language is necessary for appropriate use of the proposed product.

3.0 Introduction

3.1 Chemical Identity

Table 3.1. Demiditraz Nomenclature

Chemical Structure	
Empirical Formula	$C_{13}H_{16}N_2$
Common Name	Demiditraz
Company Experimental Names	PF-3814927
CAS Name	2-[(1S)-1-(2,3-Dimethylphenyl)ethyl]-1*H*-imidazole
CAS Registry Number	944263-65-4
End Use Product/EP	1007-OT, 14.4% SC
Chemical Class	Acaricide
Known Impurities of Concern	None

3.2 Physical/Chemical Characteristics

The physicochemical properties of the technical grade of demiditraz are summarized in Appendix B, Table B.1. The log of the octanol/water partition coefficient is 2.8, which indicates no special concern for bioaccumulation in lipophilic matrices. Technical grade demiditraz is a solid at room temperature and, therefore, the volatility of the chemical was not determined as it is not anticipated to have significant volatility.

3.3 Pesticide Use Pattern

The proposed spot-on product (EPA Reg. No. 1007-OT) has been formulated by Pfizer Animal Health for the control of fleas and ticks on dogs and puppies ages 8 weeks and older. The liquid product contains 14.4% demiditraz and 4.8% fipronil and is available in four dog weight ranges: small, 11 to 20 lbs (1.0 ml); medium, 21 to 33 lbs (1.5 ml); intermediate, 34 to 50 lbs (3.0 ml); and large, 51 to 66 lbs (4.0 ml). The spot-on product is not proposed for use on cats. Details of the proposed use are summarized in Table 3.3.

Table 3.3. Summary of Proposed Demiditraz Spot-On Use

EPA Reg. No.	Use Site	Application Rate	Use Restrictions
1007-OT (14.4% ai)	Small dogs from 11 to 20 lbs	0.00032 lb ai; 150 mg ai	A single dose of 1007-OT is effective up to 6 weeks in controlling fleas and up to 4 weeks for ticks. Monthly application is recommended for effective control of fleas and ticks and to prevent infestation.
	Medium dogs from 21 to 33 lbs	0.00050 lb ai; 230 mg ai	
	Intermediate dogs from 34 to 50 lbs	0.00099 lb ai; 450 mg ai	
	Large dogs from 51 to 66 lbs	0.0013 lb ai; 600 mg ai	

3.4 Anticipated Exposure Pathways

The Registration Division (RD) has requested an assessment of human health risk to support the proposed new use of demiditraz as a dog spot-on product. Humans may be exposed to demiditraz from occupational and/or residential use of the proposed product. Exposures may occur from occupational and residential adult handlers applying the spot-on product to dogs, as well as from adult and child post-application contact with treated dogs. Because there are no proposed or existing food uses for demiditraz, no dietary or drinking water exposures are anticipated.

3.5 Consideration of Environmental Justice

Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment, in accordance with U.S. Executive Order 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," (http://www.eh.doe.gov/oepa/guidance/justice/eo12898.pdf. As a part of every pesticide risk assessment, OPP considers a large variety of consumer subgroups according to well-established procedures. In line with OPP policy, HED estimates risks to population subgroups from pesticide exposures that are based on patterns of that subgroup's food and water consumption, and activities in and around the home that involve pesticide use in a residential setting. Extensive data on food consumption patterns are compiled by the USDA under the Continuing Survey of Food Intake by Individuals (CSFII) and/or the CDC under the National Health and Nutrition Examination Survey/What We Eat in America (NHANES/WWEIA), and are used in pesticide risk assessments for all registered food uses of a pesticide. These data are analyzed and

categorized by subgroups based on age, season of the year, ethnic group, and region of the country. Additionally, OPP is able to assess dietary exposure to smaller, specialized subgroups and exposure assessments are performed when conditions or circumstances warrant. Whenever appropriate, non-dietary exposures based on home use of pesticide products and associated risks for adult applicators and for toddlers, youths, and adults entering or playing on treated areas post-application are evaluated. Further considerations are currently in development as OPP has committed resources and expertise to the development of specialized software and models that consider exposure to bystanders and farm workers as well as lifestyle and traditional dietary patterns among specific subgroups.

4.0 Hazard Characterization and Dose-Response Assessment

4.1 Summary of Toxicological Effects

The nervous system is the primary target organ for demiditraz. Evidence of neurotoxicity after demiditraz exposure was observed in the following toxicity studies: acute neurotoxicity (ACN); subchronic neurotoxicity (SCN); developmental neurotoxicity (DNT); subchronic dermal in rats; subchronic oral in dogs; 2-generation reproduction in rats; and rat and rabbit developmental. Demiditraz effects on the nervous system were manifested as the following clinical signs which were observed within 30 minutes of dosing: altered gait and posture; impaired mobility; decreased rearing, in-coordination; increased urination; lower body temperature; subdued appearance; rocks; lurches and sways when walking; hunched posture; hypoactivity; sitting with the head held low; a flattened body; piloerection; tremors/convulsions; prostration; wet and yellow urogenital area; shallow and/or decreased respiration; lacrimation; salivation; and dilated pupils. Decreased motor activity was noted in both sexes in the ACN and dermal toxicity studies, whereas increased motor activity was observed in the SCN study. In the DNT, decreases and increases in motor activity were observed in the offspring at various ages; reduced grip strength, and increased amplitude and reduced latency were observed in the startle response test. Although the latter findings may be related to a general delay in offspring development rather than to neurotoxicity, they are consistent with the findings in other demiditraz toxicity studies.

In the developmental toxicity studies, maternal toxicity was observed in the rat and rabbit, as evidenced by mortality, clinical signs of toxicity, and decreased body weight. Developmental toxicity was observed in the rat and rabbit, as evidenced by decreased fetal body weight and associated delayed ossification of the skeleton in rats and slight increases in the incidence of 27th presacral vertebrae and 13th full ribs in the rabbit. In the rat reproductive toxicity study, mortality, clinical signs, and decreased body weight were observed in parental rats, and decreased survival, decreased brain weight, and decreased pup body weight were observed in the offspring. Reproductive toxicity was not observed. In the developmental neurotoxicity study in rats, maternal toxicity was observed, as evidenced by clinical signs of toxicity and effects on maternal behavior. The effects observed in the offspring included a delay in preputial separation in males and changes in motor activity, startle response, and grip strength, which may be attributed to a general delay in development.

The mutagenicity/genetic toxicity database consists of a bacterial reverse mutation test (Ames test), a forward mutation test in the V79/HGPRT test, chromosome aberration test in Chinese

Hamster Ovary cells, and a mouse bone marrow micro nucleolus test. All studies were determined to be negative for mutagenicity and/or genetic toxicity, and there is no concern for mutagenicity/genetic toxicity for demiditraz. Demiditraz was assessed in a guideline immunotoxicity study, and no specific immunotoxicity was identified.

In an absorption and distribution study in rats, evidence was obtained that showed a significant portion of the administered dose to the maternal rat partitioned into the milk and was available to the F1 offspring through nursing. The amount of test material in the stomach of the offspring was similar to the concentration in maternal milk. Detectable levels of the test material were observed in neonatal plasma demonstrating exposure to the offspring following maternal exposure, which negated the need for direct exposure of the offspring in the developmental neurotoxicity study.

Based on limited metabolism data, demiditraz was rapidly absorbed and eliminated via the urine (71%) and feces (21%), with 92% being excreted within 48 hours post dose in the rat. The half-life in rats following oral exposure was determined to be approximately 30 minutes. In the dog, the half-life was 4.7 hours (oral) and 347 hours (dermal), and bioavailability was 10.1% following oral exposure and 18.6% following dermal exposure.

A dermal absorption study is available for demiditraz, which identified a dermal absorption factor of 30%. Since the dermal risk assessments for all durations of exposure are based on a route-specific study (subchronic dermal toxicity study in rats), a dermal absorption factor is not needed.

There is no subchronic inhalation toxicity study currently available to determine the potential for demiditraz to affect the nervous system by this route of exposure. It should be noted, however, that an inhalation study is not relevant to the exposure pattern anticipated for the pet use, since significant inhalation exposure is not expected.

4.2 Considerations of Toxicity to Children

Demiditraz does not require food tolerances and is considered to be a non-food/non-feed use chemical; therefore, it is not subject to the amendments to the Federal Food, Drug, and Cosmetic Act (FFDCA) promulgated under the Food Quality Protection Act (FQPA) of 1996. However, HED has assessed the potential for increased susceptibility for infants and children, and determined that no additional uncertainty factor is required for the protection of children based on considerations summarized in Sections 4.2.1 through 4.2.4, below.

4.2.1 Completeness of the Toxicology Database

The toxicology database for demiditraz is complete for the pet use being assessed. The following studies have been submitted and found to be acceptable: rat and rabbit developmental toxicity studies; rat 2-generation reproduction; rat acute and subchronic neurotoxicity; rat developmental neurotoxicity; and rat immunotoxicity study.

4.2.2 Evidence of Neurotoxicity

There is evidence of neurotoxicity throughout the database, but neuropathology was not observed. Altered gait and posture, impaired mobility, decreased rearing, in-coordination, lower body temperature, and decreases in motor activity were observed in both sexes in the acute neurotoxicity study in rats. Several clinical signs of neurotoxicity, which included subdued appearance, rocks/lurches/sways when walking, hunched posture, hypoactivity, wet and yellow urogenital area, shallow and/or decreased respiration, lacrimation, and dilated pupils, and increased motor activity were observed in both sexes in the subchronic neurotoxicity study in rats. Decreased motor activity and altered grooming behavior were observed in both sexes in the 90-day dermal toxicity study in rats. In the developmental toxicity studies, clinical signs suggestive of neurotoxicity were observed in the maternal rats (rocking, lurching, or swaying while walking, piloerection, dilated pupils, and subdued appearance) and rabbits (tremors, prostration, and/or clonic convulsions). In the 2-generation reproduction study in rats, clinical signs (subdued appearance, rocks/lurches/sways while walking, hypoactivity, tremors, clonic convulsions) suggestive of neurotoxicity were observed. In the developmental neurotoxicity study in rats, decreased/increased motor activity and increased startle response amplitude and reduced latency were observed at the high dose where a delay in overall development was observed, mainly in males, as evidenced by decreased body weights throughout lactation in both sexes, and a delay in preputial separation.

4.2.3 Evidence of Sensitivity/Susceptibility in the Developing or Young Animal

There is no evidence of increased susceptibility following *in utero* exposure to demiditraz in either the rat or rabbit developmental toxicity study, and there is no evidence of increased susceptibility following *in utero* and/or pre-/post-natal exposure in the 2-generation reproduction study in rats or in the developmental neurotoxicity study in rats.

Demiditraz has been evaluated for potential developmental effects in the rat and rabbit (gavage administration). Maternal toxicity was observed in the rat and rabbit, as evidenced by mortality and clinical signs of toxicity and decreased body weights. Developmental toxicity was observed in the rat and rabbit, as evidenced by decreased fetal body weight and associated delayed ossification of the skeleton in rats and slight increases in the incidence of 27^{th} presacral vertebrae and 13^{th} full ribs in the rabbit. The developmental effects were observed at the same dose where maternal effects were observed in the rat and at a higher dose than where maternal effects were observed in the rabbit. In the rat reproductive toxicity study, mortality, clinical signs (subdued appearance, rocks/lurches/sways while walking, hypoactivity, tremors, clonic convulsions), and decreased body weight were observed in parental rats, and decreased survival, decreased brain weight, and decreased pup body weight were observed in the offspring at the same dose levels. In the developmental neurotoxicity study in rats, maternal toxicity (sitting with head held low, hypoactivity, a flattened body, slightly drooping eyelids, decreased respiration, clear material around mouth/salivation, lacrimation, and/or dilated pupils) was observed at a dose where no effects were observed in the offspring.

4.2.4 Residual Uncertainty in the Exposure Database

There is no residual uncertainty in the exposure database. Dietary (food and water) exposure assessments are not applicable to this assessment. Chemical-specific data are available for assessing exposure resulting from spot-on treatments of demiditraz; these data will not underestimate residential exposure.

4.3 Toxicity Endpoint and Point of Departure Selections

4.3.1 Dose-Response Assessment

A detailed description of the toxicity studies used for toxicity endpoints and points of departure for various exposure scenarios is presented in Appendix A.

No acute or chronic dietary assessments are required since demiditraz is a non-food use pesticide.

The incidental oral endpoint (all durations) for risk assessment was from the oral subchronic neurotoxicity study in the rat with a NOAEL of 5 mg/kg/day. At the study LOAEL of 25 mg/kg/day, clinical signs of neurotoxicity (subdued appearance, rocks, lurches, or sways when walking, hunched posture, hypoactivity, shallow/decreased respiration, lacrimation, dilated pupil), decreased motor activity, and decreased body weights were observed in both sexes. Inhalation hazard is assumed to be equivalent to oral hazard.

The current risk assessment includes a chronic exposure assessment. The Point of Departure for assessing risks resulting from this duration of exposure was also the NOAEL from the subchronic neurotoxicity study. This 90-day duration POD is acceptable for quantifying chronic risks for the long-term incidental oral and inhalation risk assessments because there is no evidence of cumulative toxicity; i.e., rapid onset (within 30 minutes of exposure) and short duration (2 hours) of transient clinical signs.

Dermal endpoints (all durations) for risk assessment were from the route-specific subchronic (90-day) dermal toxicity study in the rat with a LOAEL of 100 mg/kg/day. A NOAEL was not identified. Alterations in motor activity and grooming behavior were observed at all dose levels. The same dermal study and endpoints are appropriate for long-term dermal assessment because there is no evidence of cumulative toxicity.

An additional uncertainty factor of 3X is applied for the lack of a NOAEL ($UF_{LOAEL \rightarrow NOAEL}$). The 3X factor is considered appropriate for dermal exposure assessment (all durations). Application of the 3X factor results in an extrapolated dermal NOAEL of 33 mg/kg/day. Based on a dermal absorption factor of 31%, the orally equivalent dose is about 10 mg/kg/day. This is consistent with the 2-generation reproduction study NOAEL of 7.5 mg/kg/day, which noted similar effects as observed in the dermal study at an oral dose of 50 mg/kg/day.

The UF_{DB} of 10X retained in the previous human health risk assessment was removed since the data requirements for a rat developmental neurotoxicity study and a dog subchronic oral toxicity

study have been satisfied, and the dog was not found to be more sensitive than the rat to the neurotoxic effects of demiditraz. The demiditraz database is now considered complete.

4.3.2 Recommendation for Combining Routes of Exposures for Risk Assessment

Since the dermal and oral endpoints are based on the same effects (neurotoxicity) these routes of exposure may be combined for purposes of this risk assessment.

4.3.3 Cancer Classification and Risk Assessment Recommendation

Demiditraz has not been classified for carcinogenic potential. Rat and mouse carcinogenicity studies with demiditraz have not been submitted and are not required for the current non-food/non-feed use. All studies conducted for mutagenicity/genetic toxicity were determined to be negative and, therefore, there is no concern for mutagenicity/genetic toxicity for demiditraz. There are also no reproductive toxicity concerns.

4.3.4 Summary of Points of Departure and Toxicity Endpoints Used in Human Risk Assessment

Table 4.3.4.1. Summary of Toxicological Doses and Endpoints for Demiditraz for Use in Non-Occupational Human Health Risk Assessments

Exposure/ Scenario	Point of Departure	Uncertainty Factors	Level of Concern for Risk Assessment	Study and Toxicological Effects
All dietary Exposures	There are no current registrations for food uses. Therefore, no dietary endpoint has been selected for demiditraz at this time.			
Dermal (All Durations)	LOAEL = 100 mg/kg/day	UF_A= 10X UF_H= 10X UF_{LOAEL}= 3X	MOE = 300	Subchronic dermal toxicity study LOAEL = 100 mg/kg/day, based on decreased motor activity in males and indications of ungroomed appearance. A NOAEL was not demonstrated since there was toxicity (decreased motor activity) and ungroomed appearance at the lowest dose tested.
Incidental Oral (All Durations)	NOAEL= 5 mg/kg/day	UFA= 10X UFH= 10X	MOE = 100	Subchronic neurotoxicity study with a LOAEL = 25 mg/kg based on clinical signs of neurotoxicity.

Table 4.3.4.1. Summary of Toxicological Doses and Endpoints for Demiditraz for Use in Non-Occupational Human Health Risk Assessments				
Exposure/ Scenario	**Point of Departure**	**Uncertainty Factors**	**Level of Concern for Risk Assessment**	**Study and Toxicological Effects**
Inhalation (All Durations)	NOAEL= 5 mg/kg/day	UF_A= 10X UF_H= 10X	MOE = 100	Subchronic neurotoxicity study with a LOAEL = 25 mg/kg, based on clinical signs of neurotoxicity.
Cancer (oral, dermal, inhalation)	There are no carcinogenicity studies with demiditraz. The carcinogenic potential of demiditraz has not been evaluated.			

Point of Departure (POD) = A data point or an estimated point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). UF_{LOAEL} = additional factor for extrapolation from LOAEL to NOAEL. MOE = margin of exposure. LOC = level of concern. N/A = not applicable.

Table 4.3.4.2. Summary of Toxicological Doses and Endpoints for Demiditraz for Use in Occupational Human Health Risk Assessments				
Exposure/ Scenario	**Point of Departure**	**Uncertainty Factors**	**Level of Concern for Risk Assessment**	**Study and Toxicological Effects**
Dermal (All Durations)	LOAEL ≤ 100 mg/kg/day	UF_A= 10X UF_H= 10X UF_{LOAEL}= 3X	MOE = 300	Subchronic dermal toxicity study LOAEL = 100 mg/kg/day, based on decreased motor activity in males and indications of ungroomed appearance. A NOAEL was not demonstrated since there was toxicity (decreased motor activity) and ungroomed appearance at the lowest dose tested.
Inhalation (All Durations)	NOAEL = 5 mg/kg/day	UF_A= 10X UF_H= 10X	MOE = 100	Subchronic Neurotoxicity study LOAEL = 25 mg/kg, based on clinical signs of neurotoxicity.
Cancer (oral, dermal, inhalation)	There are no carcinogenicity studies with demiditraz. The carcinogenic potential of demiditraz has not been evaluated.			

Point of Departure (POD) = A data point or an estimated point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in

sensitivity among members of the human population (intraspecies). UF_{LOAEL} = extrapolation from LOAEL to NOAEL. MOE = margin of exposure. LOC = level of concern.

5.0 Residential (Non-Occupational) Exposure/Risk Characterization

Residential exposure is expected from the proposed demiditraz dog spot-on use. Residential handler exposures are anticipated from application of the proposed product to dogs, and residential post-application exposures are expected to occur from contact with dogs previously treated with demiditraz. In assessing these exposures, the *Health Effects Division's (HED) 2012 Standard Operating Procedures (SOPs) for Residential Pesticide Exposure Assessment: Treated Pets*[2] was used. Some of the data included in the 2012 Treated Pet SOP are proprietary and, thus, are subject to the data protection provisions of FIFRA.

5.1 Residential Handler Exposure

HED uses the term "handlers" to describe those individuals who are involved in the pesticide application process. HED believes that there are distinct tasks related to applications and that exposures can vary depending on the specifics of each task. Residential handlers are addressed somewhat differently by HED as homeowners are assumed to complete all elements of an application without use of any protective equipment.

Residential handler dermal exposure is expected to occur from application of the proposed demiditraz spot-on product to dogs. Inhalation exposure of spot-on products is considered to be negligible. A short-term residential handler dermal exposure assessment was performed for homeowners applying the proposed demiditraz products to dogs. Intermediate- and long-term exposures are not likely because of the intermittent nature (i.e., once monthly) of pet treatment by homeowners.

No chemical-specific unit exposure data were provided in support of this submission. Therefore, HED used exposure values from the 2012 Residential SOPs (Treated Pets) as a surrogate to estimate handler exposures. Exposure data for spot-on applications (MRID 44433303) were used to estimate handler exposures for the proposed demiditraz spot-on product.

The algorithms and inputs used to estimate exposure and dose for residential handlers can also be found in the occupational and residential exposure and risk assessment[3] document which supports this memorandum.

Residential handler short-term dermal risk estimates are not of concern (i.e., MOEs > 300) for all dog weight ranges proposed. A summary of residential handler risk estimates is presented in Table 5.1.

2 http://www.epa.gov/pesticides/science/residential-exposure-sop.html

[3] W. Britton. Demiditraz: Occupational and Residential Exposure and Risk Assessment for the Proposed Pet Spot-On Use (1007-OT). D400484,.D409676, D409677. 4/11/13.

Table 5.1. Residential Handler Short-Term Non-cancer Exposure and Risk Estimates from Use of the Proposed Demiditraz Spot-On

Exposure Scenario	Reg. No.	Level of Concern	Dermal Unit Exposure (mg/lb ai)	Inhalation Unit Exposure (mg/lb ai)	Maximum Application Rate[1] (lb ai/pet)	Amount Handled Daily[2]	Dermal	
							Dose (mg/kg/day)[3]	MOE[4]
Applying Demiditraz Spot-on	1007-OT (14.4% ai)	Dermal: 300	120	Negligible	0.00032 (small)	2 Dogs	0.00097	100,000
					0.00050 (medium)		0.0015	67,000
					0.00099 (intermediate)		0.0030	34,000
					0.0013 (large)		0.0040	25,000

1 Based on registered or proposed label (EPA Reg. No.1007-OT).
2 Based on HED's 2012 Residential SOPs (Treated Pets) (http://www.epa.gov/pesticides/science/residential-exposure-sop.html).
3 Dermal Dose = Dermal Unit Exposure (120 mg/lb ai) × Application Rate (lb ai/ treatment) × Area Treated or Amount Handled (2 Dogs/day) × Dermal Absorption Factor (1.0) ÷ Body Weight (80 kg).
4 Dermal MOE = Dermal NOAEL (100 mg/kg/day) ÷ Dermal Dose (mg/kg/day).

5.2 Residential Post-Application Exposure

There is the potential for post-application exposure for individuals exposed as a result of contacting a dog previously treated with the proposed demiditraz spot-on product. The quantitative exposure/risk assessment for residential post-application exposures is based on the following scenarios:

1) Post-application dermal (adults and children 1 to < 2 years old) exposure from contacting dogs treated with demiditraz; and
2) Post-application incidental oral exposure (children 1 to < 2 years old only) from contacting dogs treated with demiditraz.

The lifestages selected for each post-application scenario are based on an analysis provided as an Appendix in the 2012 Residential SOPs[4]. These lifestages are not the only that could be potentially exposed for these post-application scenarios; however, the assessment of these lifestages is health protective for the exposures and risk estimates for any other potentially exposed lifestages.

Due to the preventative nature of pet products and the potential for extended usage in more temperate parts of the country, the potential exists for applications to extend beyond a short-term duration. Therefore, residential post-application exposures of short-, intermediate-, and long-term durations were assessed for the proposed demiditraz spot-on product.

[4] Available: http://www.epa.gov/pesticides/science/residential-exposure-sop.html

A series of assumptions and exposure factors served as the basis for completing the residential post-application risk assessment. Each assumption and factor is detailed in the 2012 Residential SOPs. A chemical-specific pet fur residue transfer study (MRID 48766704) was submitted by Pfizer Animal Health in support of the proposed demiditraz spot-on use. HED has reviewed the exposure study[5] and this data was determined to acceptable for use for assessment of exposure and risk from the proposed use.

The 2012 Residential SOPs (Treated Pets) recommends assessment of post-application exposures using day of application (i.e., Day 0) residue transfer -- defined as fraction application rate (F_{AR}) in the 2012 SOPs. HED used the average of Day 0 percent residue transfer values predicted from regression analysis in order to assess exposures and risks for all durations of post-application exposures from the proposed demiditraz spot-on use. Day 0 F_{AR} was determined to be 2.8%.

A refined approach is also presented for the assessment of longer-term (i.e., intermediate- and long-term) post-application exposures. Per the 2012 Treated Pet SOP, the assessment of post-application exposures to treated pets can be refined to more accurately reflect exposures over longer periods of time (e.g., several months). Instead of using the Day 0 percent residue transfer value, the average of percent residue transfer values predicted from Days 0 to 30 (i.e., proposed product re-treatment interval) was inputted. The F_{AR} for the 30 day interval is 0.38%.

HED combines risk values resulting from separate routes of exposure when the hazard associated with the points of departure is similar across routes. A common toxicological endpoint, neurotoxicity, exists for dermal and incidental oral routes of exposure to demiditraz. Residential post-application inhalation exposure is expected to be negligible from the proposed spot-on product and, thus, a quantitative assessment was not performed.

Residential post-application adult dermal, and combined child 1 to < 2 years old years old exposures (all durations) are not of concern (i.e., adult dermal MOEs are > 300; and children 1 to < 2 years old year old ARIs are > 1) with use of Day 0 F_{AR}. Exposures estimated for the longer-term exposures using 30 day average residue data are approximately 7X below (MOEs 7X greater) those estimated for all durations using Day 0 F_{AR}.

Table 5.2. Residential Post-Application Non-Cancer Exposure and Risk Estimates from the Proposed Demiditraz Spot-On Product: All Durations (Day 0 F_{AR})

Lifestage	Post-application Exposure Scenario		Application Rate (mg ai)[1]	Dose (mg/kg/day)[2]	MOEs[3]	ARI[4]
	Use Site	Route of Exposure				
Adult	Dog	Dermal	150 (small)	0.066	1,500	NA
			230 (medium)	0.067	1,500	
			450 (intermediate)	0.096	1,000	
			600 (large)	0.099	1,000	

[5] W. Britton, Demiditraz: Data Evaluation Record for the Study "Determination of Transferable Residues of Demiditraz and Fipronil from the Hair of Dogs Following the Spot-on Treatment Separately with Three Different Formulated End-Use Products." D409400, EPA MRID 48766704, 4/11/13.

Table 5.2. Residential Post-Application Non-Cancer Exposure and Risk Estimates from the Proposed Demiditraz Spot-On Product: All Durations (Day 0 F_{AR})						
Lifestage	**Post-application Exposure Scenario**		**Application Rate (mg ai)[1]**	**Dose (mg/kg/day)[2]**	**MOEs[3]**	**ARI[4]**
	Use Site	**Route of Exposure**				
Children 1 to < 2 Years Old		Dermal	150 (small)	0.17	600	1.9
		Incidental Oral		0.0017	3,000	
		Dermal	230 (medium)	0.17	590	1.8
		Incidental Oral		0.0017	2,900	
		Dermal	450 (intermediate)	0.25	410	1.3
		Incidental Oral		0.0025	2,000	
		Dermal	600 (large)	0.25	400	1.3
		Incidental Oral		0.0025	2,000	

1 Based on proposed label (Reg. No. 1007-OT)

2 Dermal Dose = [(Transfer Coefficient (cm^2/hr) * (Application Rate (150, 230, 450, 600 mg ai) * Fraction Application Rate (0.028) ÷ Surface Area of Dog (cm^2)) * Exposure Time (hours/day) * Absorption Factor (unitless)) / Body Weight (80 kg, adult; 11 kg, child 1 to < 2 years old years old)]
Hand-to-Mouth Dose = [(Hand Residue Loading (mg/cm^2) × Fraction of Hand Mouthed (0.13) × Surface Area of 1 Hand (150 cm^2) × Exposure Time (1.5 hrs/day) × # of Replenishment Intervals/hr (4 int/hr) × (1-((1-Saliva Extraction Factor (0.5))^(Number of Hand-to-Mouth Events per Hour (13.9 events/hr)) ÷ (# of Replenishment Intervals/hr))] / Body Weight (11 kg child 1 to < 2 years old years old)]

3 MOE = Dermal, Incidental Oral POD (mg/kg/day) / Dose (mg/kg/day)

4. ARI = 1÷ [(1/ (Dermal MOE/Dermal LOC; 300)) + (1/(Incidental Oral MOE÷Incidental Oral LOC; 100))]

5.3 Combined Residential Risk Estimates (Multiple Exposure Scenarios)

Residential handler and post-application scenarios should generally not be combined. There is the potential for the same individual (i.e., adult) to apply a pesticide in and around the home and be exposed by reentering a treated area in the same day; however, combining both of these exposure scenarios would be inappropriate because of the conservative nature of each individual assessment.

5.4 Residential Risk Estimates for Use in Aggregate Assessment

An aggregate exposure assessment, which combines exposures from different sources and routes, is typically conducted for food/feed chemicals when there is potential for human exposure through drinking water and residential pathways. Demiditraz currently has no registered food/feed uses and no drinking water residues are expected to result from the proposed use; therefore, the aggregate exposure assessment consists only of the proposed use.

5.5 Residential Bystander Post-Application Inhalation Exposure

Occupational and residential inhalation exposures from dog spot-on treatments are considered negligible.

6.0 Aggregate Exposure/Risk Characterization

The demiditraz dog spot-on product is classified as a non-food/non-feed use with no proposed or existing food uses. Because demiditraz currently has no registered food uses and no drinking water residues are expected to result from the proposed pet use, the aggregate exposure assessment consists only of exposures from the proposed use.

7.0 Cumulative Exposure/Risk Characterization

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to demiditraz and any other substances and demiditraz does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that demiditraz has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative.

8.0 Occupational Exposure/Risk Characterization

Occupational handler exposures are anticipated from the application of the proposed demiditraz spot-on product. Occupational post-application activities are expected to be significantly less than residential post-application exposures.

8.1 Occupational Handler Exposure/Risk Estimates

Based on the anticipated use patterns and current labeling, types of equipment and techniques that can potentially be used, occupational handler exposure (i.e., veterinarians, veterinary assistants, and groomers) could be expected from the demiditraz spot-on use. The quantitative exposure/risk assessment developed for occupational handlers (adults) is based on dermal exposure from the application of the spot-on product to dogs. Inhalation exposures are expected to be negligible from application of the spot-on product.

A series of assumptions and exposure factors served as the basis for completing occupational handler risk assessments. A number of these are the same used for assessment of residential handler exposures. An assumption unique to the assessment of occupational handler exposures for the proposed demiditraz dog use is the number of animals treated daily. There is currently no recommendation for the number of animals treated by occupational handlers per application event. For the purpose of assessing occupational handler risk, the maximum number of dogs that could be treated with the proposed spot-on product without resulting in a risk of concern has been presented.

Similar to residential use of the proposed spot-on product, occupational exposures are also expected to extend beyond a short-term duration due to temperate climates in some parts of the

country and the preventative nature of spot-on pet products. For these reasons, it is expected that veterinarians, veterinary assistants, and groomers could treat dogs with the proposed demiditraz spot-on product for all durations of exposure (short-, intermediate-, and long-term) for flea and tick prevention and/or treatment.

The algorithms and inputs used to estimate exposure and dose for occupational handlers are the same as though used for residential handler exposure assessment, except for the number of pets treated explained above, and can be found in the 2012 Residential SOPs (Treated Pets) SOP, as well as in the occupational and residential exposure and risk assessment[6] which supports this memorandum.

Only dermal exposures are expected from the proposed demiditraz spot-on use and, therefore, exposures were not combined from multiple routes.

Occupational handlers of the proposed demiditraz spot-on product could treat up to 170 large dogs per day (all durations) without resulting dermal risks of concern (i.e., MOEs are ≥ 300). It is highly unlikely that this large a number of dogs could be treated daily in an occupational setting and, therefore, no risk concerns are anticipated from occupational use of the proposed dog spot-on product. A summary of occupational handler dermal risk estimates is presented in Table 8.1.

[6] W. Britton. Demiditraz: Occupational and Residential Exposure and Risk Assessment for the Proposed Pet Spot-On Use (1007-OT). D409676.D409677. 4/11/13.

Table 8.1. Occupational Handler Non-Cancer Exposure and Risk Estimates (All Durations) for Demiditraz									
Exposure Scenario	**Target**	**Dermal Unit Exposure (mg/lb ai)[1]**	**Inhalation Unit Exposure (mg/lb ai)**	**Maximum Application Rate (lb ai)[2]**	**Maximum Amount Handled Daily[3]**	**Dermal**		**Inhalation**	
		Single Layer, No Gloves	**No Respirator**			**Dose (mg/kg/day)[4]**	**MOE[5]**	**Dose (mg/kg/day)**	**MOE**
Applying Demiditraz Spot-On Product	Dogs	120	Negligible	0.00032 (small)	170 Dogs	0.082	1,200	Negligible	
				0.00050 (medium)		0.13	800		
				0.00099 (intermediate)		0.25	400		
				0.0013 (large)		0.34	300		

1 Based on the "Occupational Pesticide Handler Unit Exposure Surrogate Reference Table" (March 2012); Level of mitigation: Baseline
2 Based on registered or proposed label (Reg. No. 1007-OT)
3. Maximum number of dogs that could be treated per day and not result in risks of concern (i.e., short-, intermediate and long-term, MOEs = 300)
4 Dermal Dose = Dermal Unit Exposure (120 mg/lb ai) × Application Rate (lb ai/ treatmentl) × Amount Handled Daily (treatments/day) × DAF (1.0) ÷ BW (80 kg)
5 Dermal MOE = Dermal NOAEL (100 mg/kg/day) ÷ Dermal Dose (mg/kg/day)

8.2 Occupational Post-Application Risk

HED uses the term post-application to describe exposures that occur when individuals are present in an environment that has been previously treated with a pesticide (also referred to as re-entry exposure). For the proposed demiditraz pet collar use, occupational post-application activities are expected to be significantly less than residential post-application exposures. That is, dogs are expected to be treated and returned to their owners such that post-application contact will be negligible. As a result, no quantitative occupational post-application exposure and risk assessment has been performed. The residential post-application exposure and risk assessment (Section 5.2) is considered protective for any potential occupational post-application exposures and risks.

References

W. Britton. Demiditraz: Human Health Risk Assessment for Proposed Dog Spot-On Use. D378783. 11/30/2010.

W. Britton. Demiditraz: Occupational and Residential Exposure and Risk Assessment for the Proposed Pet Spot-On Use (1007-OT). D409676.D409677. 4/11/13.

W. Britton. Demiditraz: Data Evaluation Record for the Study "Determination of Transferable Residues of Demiditraz and Fipronil from the Hair of Dogs Following the Spot-on Treatment Separately with Three Different Formulated End-Use Products." D409400, EPA MRID 48766704, 4/11/13.

Appendix A. Toxicology Profile and Executive Summaries

A.1 Toxicology Data Requirements

The requirements (40 CFR 158.340) for food and non food uses for demiditraz are in Table A.1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

A.1. Summary of Toxicology Data Requirements - Demiditraz

Study		Technical	
		Required	Satisfied
870.1100	Acute Oral Toxicity	yes	yes
870.1200	Acute Dermal Toxicity	yes	yes
870.1300	Acute Inhalation Toxicity	yes*	no*
870.2400	Primary Eye Irritation	yes	yes
870.2500	Primary Dermal Irritation	yes	yes
870.2600	Dermal Sensitization	yes	yes
870.3100	Oral Subchronic (rodent)	CR	yes
870.3150	Oral Subchronic (nonrodent)	CR	yes
870.3200	21-Day Dermal	no	no
870.3250	90-Day Dermal	yes	yes
870.3465	90-Day Inhalation	CR	no
870.3700a	Developmental Toxicity (rodent)	Yes	yes
870.3700b	Developmental Toxicity (nonrodent)	yes	yes
870.3800	Reproduction	yes	yes
870.4100a	Chronic Toxicity (rodent)	CR	no
870.4100b	Chronic Toxicity (nonrodent)	no	no
870.4200a	Oncogenicity (rat)	CR	no
870.4200b	Oncogenicity (mouse)	CR	no
870.5100	Mutagenicity—Gene Mutation - bacterial	Yes	yes
870.5300	Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5xxx	Mutagenicity—Structural Chromosomal Aberrations	yes	yes
870.6100a	Acute Delayed Neurotoxicity (hen)	no	no
870.6100b	90-Day Neurotoxicity (hen)	no	no
870.6200a	Acute Neurotoxicity Screening Battery (rat)	yes	yes
870.6200b	90-Day Neurotoxicity Screening Battery (rat)	yes	yes
870.6300	Develop. Neurotoxicity	CR	yes
870.7485	General Metabolism	CR	no
870.7600	Dermal Penetration	CR	no
870.7800	Immunotoxicity	yes	yes

*Refer to review by R. Whiting dated May 21, 2010. There were technical problems in generating a test atmosphere that precluded completion of the acute inhalation toxicity study.
CR = conditionally required

A.2 Toxicity Profiles

Table A.2.1. Acute Toxicity Profile – Technical Demiditraz				
Guideline No.	**Study Type**	**MRID(s)**	**Results**	**Toxicity Category**
870.1100	Acute oral - rat	47744106	LD_{50} = 5000 mg/kg	III
870.1200	Acute dermal - rat	47744107	LD_{50} > 5000 mg/kg	IV
870.1300	Acute inhalation - rat	None	Waived	Technical complications in conducting the study. Test material could not be generated as an aerosol.
870.2400	Acute eye irritation - rabbit	47744109	Slight or non-irritating	IV
870.2500	Acute dermal irritation - rabbit	47744110	Slight irritant	IV
870.2600	Skin sensitization - guinea pig	47744111	Not a sensitizer	N/A

Table A.2.2. Demiditraz Study Details and Neurotoxicity Effects Observed			
Guideline No.	**Study Type**	**MRID No. (year)/ Classification Doses**	**Results**
870.6200a	Acute neurotoxicity (rat, Sprague-Dawley)	MRID 47744113 (2008) Acceptable/Guideline 0, 15, 100, 600 mkd	**NOAEL = 15 mkd.** 1 dose *via g*avage FOB/MA assessed ≈15 minutes post dose LOAEL = 100 mkd, based on altered gait and posture, impaired mobility, decreased rearing, in-coordination, increased urination, lower body temperature, decreased in motor activity in both sexes; at 600 mkd, clonic convulsions, hindlimb splay/dragging, ↓rotarod performance; low arousal.
870.6200b	Subchronic neurotoxicity (rat, Sprague-Dawley)	MRID 47744114 (2008) Acceptable/Guideline 0, 5, 25, 125 mkd	**NOAEL = 5 mkd.** 90 days *via g*avage FOB/MA assessed prior to dosing (wks 1, 3, 7, 12) LOAEL = 25 mkd, based on clinical signs (subdued appearance, rocks, lurches/sways when walking, hunched posture, hypoactivity, etc., both sexes), ↓BW, increased motor activity; 125 mkd increased incidence of same findings.
870.3150	Subchronic oral toxicity (dog, Beagle)	MRID 48459302 (2009) Acceptable/Guideline 0, 10, 30, 100 mkd	**NOAEL = 10 mkd.** 90 days capsule assessed 30 minutes to 2 hrs post dose LOAEL = 30 mkd, based on hypoactivity in both

Table A.2.2. Demiditraz Study Details and Neurotoxicity Effects Observed			
Guideline No.	**Study Type**	**MRID No. (year)/ Classification Doses**	**Results**
			sexes. At 100 mkd, all dogs displayed hypoactivity.
870.3250	Subchronic dermal toxicity (rat, Sprague-Dawley)	MRID 47752701 (2008) Acceptable/Guideline 6 hr/day for 90 days 0, 100, 300, 1000 mkd	**NOAEL not identified.** 6 hr/day for 90 days FOB/MA assessed prior to dosing during wk 10 and 20 minutes after removal of test material during wk 11 Dermal LOAEL = 100 mkd, based on decreased motor activity in both sexes at all dose levels. With 31% DAF, internal dose of 31 mkd (LOAEL)
870.3800	2-generation reproduction (rat, Sprague-Dawley)	MRID 47744117 (2009) Acceptable/Guideline Crl:CD(SD) 10 weeks pre-mating 0, 7.5, 50, 150 mkd	**NOAEL = 7.5 mkd.** 10 weeks pre-mating *via g*avage assessed ≈30 minutes post dose LOAEL = 50 mkd based on numerous clinical signs (both sexes; both generations); subdued appearance; rocks, lurches, or sways as it walks; pupils dilated; and hypoactivity; at 150 mkd, increased incidences tremors (both sexes), respiratory effects, and clonic convulsions (females), gasping (F1 male pups).
870.3700a	Developmental toxicity (rat, Sprague-Dawley)	MRID 47755402 (2008) Acceptable/Guideline GD 6-19 0, 5, 25, 100 mkd	**NOAEL = 25 mkd.** GD 6-19 (14 days) *via g*avage assessed ≈30 minutes post dose LOAEL = 100 mkd, based on mortality (1 on GD 19), clinical signs (behavioral findings of subdued appearance; rocking, lurching, or swaying while ambulating; rocking, piloerection, dilated pupils, sitting with head held low and appeared hypoactive but exhibiting normal behavior when stimulated), and wet clear material around mouth.
870.3700b	Developmental toxicity (rabbit)	MRID 47755401 (2008) Acceptable/Guideline GD 6-29 0, 10, 50, 200/150 mkd	**Maternal NOAEL = 10 mkd.** **Developmental NOAEL = 50 mkd.** GD 6-29 (24 days) *via g*avage assessed ≈30 minutes post dose LOAEL = 50 mkd, based on mortality (GD 16), clinical signs (tremors, prostration, and/or clonic convulsions); at 200 mkd, mortality GD 6, 9 @ 200 mkd, clinical signs (tremors, prostration, and/or clonic convulsions). LOAEL = 200 mkd (developmental).
870.5100	Gene Mutation Ames test	MRID 47752704 (2008) Acceptable/Guideline 0-5000µg/plate	Negative. No evidence of induced mutant colonies over background
870.5300	Forward mutation V79/HPRT	MRID 47755404 (2008) Acceptable/Guideline 0-9 µg/mL	Negative. No evidence of induced mutant colonies over background in the presence or absence of S9-activation.
870.5375	Mammalian cell cytogenetics assay	MRID 47752705 (2008) Acceptable/Guideline 0-2000 µg/mL	There was no evidence of chromosome aberrations induced over background in the presence or absence of S9-activation.
870.5395	Bone marrow micronucleus assay	MRID 47755403 (2008) Acceptable/Guideline	There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone

Table A.2.2. Demiditraz Study Details and Neurotoxicity Effects Observed			
Guideline No.	**Study Type**	**MRID No. (year)/ Classification Doses**	**Results**
		0-1000 mg/kg/day	marrow after any treatment time.
870.6300	Developmental neurotoxicity (rat, Sprague-Dawley)	MRID 48766703 (2012) Acceptable/Guideline Crl:CD(SD) GD 6 to LD 21 0, 5, 15, 100 mkd	Maternal **NOAEL = 5 mkd.** From GD 6 to LD 21 (≈40 days) *via g*avage Offspring **NOAEL = 15 mkd.** Until PND 72 *via g*avage (≈50 days) assessed ≈30 minutes and 2 hrs post dose Maternal LOAEL = 15 mkd, based on maternal clinical signs (sitting with the head held low, hypoactivity, a flattened body, slightly drooping eyelids, decreased respiration, clear material around the mouth/salivation, lacrimation, and/or dilated pupils); at 100 mkd, same as above and slightly increased post-dosing incidences of findings indicative of diminished maternal care (dam away from the nest, but not eating, drinking, grooming, or tending to the litter, and 1-3 pups outside of the nest). Offspring LOAEL = 100 mkd, based on based on decreased postnatal survival, decreased pup body weights and body weight gains, increased maximum response to the auditory startle stimulus and reduction in response time on PND 60, decreased/increased total and ambulatory motor activity counts, decreased grip strength (PND 21 and PND 35), decreased brain weight in PND 21 males, and delayed attainment of
870.7485	General metabolism	MRID 47744136 (2008) MRID 47752712 (2008) MRID 47752713 (2009) MRID 47752714 (2009) MRID 47755406 (2009)	92% of administered oral dose recovered in 48 hours; 71% (urine); 21% (feces). Excretion nearly complete (84%) in 24 hours. i.v., oral, i.p., similar half-life of 0.28 to 0.5 hour. i.v., oral, dermal half-lives of 1.06, 4.72, 347 hours; bioavailability reported to be 10.1% (oral); 18.6% (dermal).
870.7800	Immunotoxicity (rat, Sprague-Dawley)	MRID 47744122 (2009) Crl:CD(SD) 0, 10, 30, 100 mkd	via gavage (28 days) NOAEL (systemic toxicity) = 30 mkd. assessed ≈30 minutes post dose Negative for immunotoxicity potential. 100 mkd (HDT). LOAEL (systemic toxicity) = 100 mg/kg/day, based on clinical signs of toxicity (rocking lurching, or swaying while walking; drooping eyelids; subdued appearance at cage-side observation; flattened body; hunched posture; clear material around mouth; and yellow material on various body surfaces).
870.7600	Dermal absorption (rat)	MRID 47752709 (2008)	31% based on amount absorbed and remaining on skin after 168 hours.

A.3 Hazard Identification and Endpoint Selection

A.3.1 Acute and Chronic Reference Doses (aRfD and cRfD) – All Populations

Study Selected: No studies were selected. These values are calculated for dietary exposures, which currently is not a relevant route of exposure for the proposed use.

A.3.2 Incidental Oral – Short- and Intermediate-Terms

Study Selected: Combined Subchronic Oral Toxicity and Neurotoxicity Study

MRID No.: 47744114
Executive Summary: See Appendix A, Guideline [§ 870.3100 and 870.6200b]

Dose and Endpoint for Establishing aRfD: The NOAEL of 5 mg/kg/day is based on clinical signs of neurotoxicity (subdued appearance, rocks, lurches/sways when walking, hunched posture, hypoactivity, etc.) in both sexes.

UF(s): A UF of 100 was applied to account for interspecies extrapolation (10X) and intraspecies variation (10X).

Comments on Study/Endpoint/UFs: This study provides the lowest NOAEL in the database considered to be valid for this exposure and all durations and is protective for any potential adverse effects in humans. The developmental neurotoxicity study in rats provided the same NOAEL of 5 mg/kg/day in the maternal animals. The combined subchronic oral toxicity and neurotoxicity study in rats was selected in preference to the developmental neurotoxicity study because of the extensive neurotoxicity evaluations carried out in the adult male and female for up to 90 days. Most of the other oral toxicity studies gave similar values for the NOAEL: 15 mg/kg/day in the acute neurotoxicity study in rats, 10 mg/kg/day in the subchronic oral toxicity study in dogs, 7.5 mg/kg/day in the 2-generation reproduction study in rats, and 10 mg/kg/day for maternal toxicity in the developmental toxicity study in rabbits. Demiditraz acts on neurotransmitter systems in mammals. Clinical signs of neurotoxicity were the most sensitive endpoint and were observed in all the toxicity studies. The UFs included the standard accounting for interspecies extrapolation and intraspecies variation; no additional uncertainty factors were needed. There was no evidence provided which would indicate that children or more sensitive to the effects than adults.

A.3.3 Dermal – All Durations

Study Selected: Subchronic Dermal Toxicity Study

MRID No.: 47752701
Executive Summary: See Appendix A, Guideline [§ 870.3250]

Dose and Endpoint for Establishing aRfD: A NOAEL was not established. The LOAEL ≤ 100 mg/kg/day is based on decreased motor activity and ungroomed appearance.

UF(s): A UF of 300 was applied to account for interspecies extrapolation (10X), intraspecies variation (10X), and the use of a LOAEL instead of a NOAEL (3X).
Comments on Study/Endpoint/UFs: This study provides the lowest LOAEL in the database considered to be valid for this exposure and all durations and is protective for any potential adverse effects in humans. Considering that the dermal absorption is 31%, the internal dose is expected to be 31 mg/kg/day. An uncertainty factor of 3X was applied for the use of a LOAEL instead of a NOAEL. Thus, the expected internal dose for the NOAEL is 10 mg/kg/day, which is similar to the results of the oral studies. As this was the case, it was preferable to use the dermal study for the estimation of a dermal endpoint rather than an oral study. Demiditraz acts on neurotransmitter systems in mammals. Clinical signs of neurotoxicity were the most sensitive endpoint and were observed in all the toxicity studies. The UFs included the standard accounting for interspecies extrapolation and intraspecies variation, and an additional factor for using a LOAEL instead of a NOAEL (3X). There was no evidence provided which would indicate that children or more sensitive to the effects than adults.

A.3.5 Inhalation – All Durations

Study Selected: Combined Subchronic Oral Toxicity and Neurotoxicity Study

MRID No.: 47744114
Executive Summary: See Appendix A, Guideline [§ 870.3100 and 870.6200b]

Dose and Endpoint for Establishing aRfD: The NOAEL of 5 mg/kg/day is based on clinical signs of neurotoxicity (subdued appearance, rocks, lurches/sways when walking, hunched posture, hypoactivity, etc.) in both sexes.

UF(s): A UF of 100 was applied to account for interspecies extrapolation (10X) and intraspecies variation (10X). An additional factor (10X) should be applied when calculating reference doses for long-term exposure.

Comments on Study/Endpoint/UFs: This study provides the lowest NOAEL in the database considered to be valid for this exposure and all durations and is protective for any potential adverse effects in humans. No inhalation study was submitted, but inhalation exposures are not anticipated from either occupational or residential use of the spot-on product. Consequently, the oral study was selected, and complete absorption through the respiratory system was assumed. The developmental neurotoxicity study in rats provided the same NOAEL of 5 mg/kg/day in the maternal animals. The combined subchronic oral toxicity and neurotoxicity study in rats was selected in preference to the developmental neurotoxicity study because of the extensive neurotoxicity evaluations carried out in the adult male and female for up to 90 days. Most of the other oral toxicity studies gave similar values for the NOAEL: 15 mg/kg/day in the acute neurotoxicity study in rats, 10 mg/kg/day in the subchronic oral toxicity study in dogs, 7.5 mg/kg/day in the 2-generation reproduction study in rats, and 10 mg/kg/day for maternal toxicity in the developmental toxicity study in rabbits. Demiditraz acts on neurotransmitter systems in mammals. Clinical signs of neurotoxicity were the most sensitive endpoint and were observed in all the toxicity studies. The UFs included the standard accounting for interspecies extrapolation

and intraspecies variation; no additional uncertainty factors were needed, except when assessing long-term durations (10X). There was no evidence provided which would indicate that children or more sensitive to the effects than adults.

A.4 Executive Summaries

A.4.1 Sub-chronic Toxicity

870.3100a Combined Subchronic Oral Toxicity and Neurotoxicity – Rat

In a subchronic neurotoxicity study (2008, MRID 47744114), PF-3814927 (Demiditraz, 100% a.i.; Batch No. PFXU070002) in 0.5% methylcellulose and 0.1% Tween 80® was administered daily via gavage (5 mL/kg) to 22 Sprague-Dawley rats/sex/group at dose levels of 0, 5, 25, or 125 mg/kg/day for 13 weeks. Ten rats/sex/dose were designated as the Phase I (general toxicity) group, and were evaluated for hematology, clinical chemistry, ophthalmology, organ weight, and gross and histopathology parameters. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on the remaining 12 rats/sex/group (Phase II) at pre-dosing and Weeks 1, 3, 7, and 12 (prior to administration of dosing). At study termination, all Phase II animals were anesthetized and perfused *in situ* for brain weights and morphometry. The tissues from 6 rats/sex/group of the perfused animals in the control and 125 mg/kg/day groups were subjected to histopathological evaluation of central and peripheral nervous system tissues.

At 25 mg/kg/day, body weights were decreased ($p<0.05$) by 7-8% in the males during Weeks 8-13 and by 7% each in the females at Weeks 7 and 10. Body weight gains were decreased ($p<0.05$) by 33% in the males during Weeks 7-8. Overall body weight gains were decreased ($p<0.05$) by 11% in the males. At 30 minutes post-dosing, the following treatment-related clinical signs of toxicity were observed (# affected/22 vs. 0/22 controls, unless otherwise stated): (i) subdued appearance at cage-side observation only (10 males and 9 females); (ii) rocks, lurches, or sways when walking (6 males and 6 females); (iii) hunched posture (6 females); (iv) hypoactivity (2 males); (v) wet yellow material urogenital area (3 males and 6 females); (vi) lacrimation right/left eye (8 males and 19 females vs. 2 control females); (vii) dilated pupil right/left eye (5 males and 6 females); (viii) dried red material around mouth (4 males and 1 female vs. 1 control male); and (ix) wet red material around mouth (2 males). Cumulative ambulatory activity was *increased* ($p<0.05$) by 41-45% in the males at Weeks 3 and 7.

At 125 mg/kg/day, body weights were decreased ($p<0.01$) by 9-14% in the males and by 7-10% in the females during Weeks 3-13. Additionally, body weight gains were sporadically decreased ($p<0.05$) by 14-48% in the males and by 17-56% in the females. Overall (Weeks 0-13) body weight gains were decreased ($p<0.01$) by 15-19% in both sexes. At 30 minutes post-dosing, the following treatment-related clinical signs of toxicity were observed (# affected/22 vs. 0/22 controls, unless otherwise stated): (i) subdued appearance at cage-side observation only (all males and females); (ii) rocks, lurches, or sways when walking (all males and females); (iii) hunched posture (17 males and all females); (iv) hypoactivity (11 males and 3 females); (v) wet yellow material urogenital area (20 males and 21 females); (vi) shallow respiration (7 males and 1 female); (vii) decreased respiration (10 males); (viii) lacrimation right/left eye (20 males and all females vs. 2 control females); (ix) dilated pupil right/left eye (20 males and 19 females); (x)

dried red material around mouth (9 males and 11 females vs. 1 control male); and (xi) wet red material around mouth (8 of each sex). Cumulative (session) total activity was *increased* by 29-32% in the males at Weeks 1 and 3 and by 31-32% in the females throughout the study. Also at this dose, cumulative ambulatory activity was increased by 43-56% in the males at Weeks 1, 3, and 7 and by 49-65% in the females throughout the study.

No compound-related effects were observed in mortality, food consumption, ophthalmoscopic examinations, hematology, clinical chemistry, FOB parameters (note: FOB assessments were made prior to daily dosing), brain weight and morphology, organ weights, gross lesions, or microscopic lesion in either sex.

The LOAEL was 25 mg/kg/day based on multiple clinical signs of toxicity in both sexes, decreased body weight gains in males, and increased motor activity in males. The NOAEL is 5 mg/kg/day.

The study is classified as acceptable/guideline and satisfies the guideline requirement (OPPTS 870.6200b; OECD 424) for a subchronic neurotoxicity study in rats.

870.3150 90-Day Oral Toxicity – Dog

In a subchronic 90-day oral toxicity study (2009, MRID 48459302) demidiraz (99.8% a.i., Lot # TCK08001K) was administered to 4 beagle dogs/sex orally in gelatin capsules at dose levels of 0 (control), 10, 30 or 100 mg/kg bw/day. Clinical signs of toxicity, mortality, ophthalmologic, hematology, clinical chemistry, urinalysis, electrocardiographic measurements, pathology and biokinetic data were obtained.

There were no adverse effects on mortality, ophthalmology, hematology, clinical chemistry urinalysis and electrocardiographic examinations. In the 30 mg/kg/day hypoactivity was observed in 1 male and 1 female, and in all 8 dogs in the 100 mg/kg/day group. There was an increased incidence of vomitus containing food in 2 males and 2 females in the 30 mg/kg/day group and in 4 males and 3 females in the 100 mg/kg/day group. Body weight gain was statistically significantly decreased in males in the 100 mg/kg/day group from predosing day 17 to Day 91 by 20% . Females in the 100 mg/kg day group from predosing day 17 to Day 91showed a loss of weight of 0.2 kg. Food consumption was statistically significantly decreased in males in the 100 mg/kg/day group from 36-80%. Three of 4 males in the 100 mg/kg/day group exhibited treatment related minimal cell infiltrates in the adrenal zona fasiculata.

The LOAEL is 30 mg/kg bw/day in males and females, based on behavioral toxicity; i.e., an increased incidence of hypoactivity. The NOAEL in males and females is 10 mg/kg bw/day.

Pharmacokinetic data indicated that demiditraz did not bioaccumulate and there were no sex differences in the pharmacokinetics.

This 90-day oral toxicity study in the dog is acceptable/guideline and satisfies the requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in the dog.

870.3250 90-Day Dermal Toxicity – Rat

In a repeated-dose dermal toxicity study (2008, MRID 47752701), PF-3814927 (Demiditraz, ≥99.5% a.i., Lot #s PFXA070001 and PFXU070002) in deionized water was applied to the shaved intact skin of 10 Sprague-Dawley rats/sex/dose at dose levels of 0, 100, 300, or 1000 mg/kg/day (limit dose), 6 hours/day for 91/92 consecutive days. Additional groups of 3 rats/sex/dose were exposed at the same doses for 87 consecutive days. Blood was collected from the latter animals on Days 0 and 86 (approximately 6, 12 and 24 hours after application of the test material and the plasma was used for toxicokinetic exposure evaluations. Systemic exposure (as assessed by C_{max} and AUC_{0-24}) "generally increased or remained unchanged with increasing dose." For example, at day 87 the mean plasma concentration (ng/ml) was 479±743, 1150±1540 and 1270±715 for the 100, 300 and 1000 mg/kg/day dose groups, respectively. There is an apparent increase between the lowest dose and middle dose but the two highest doses are closer together.

Clinical signs were consisted of increased incidences of wet and/or dried yellow material on various body surfaces and dried red material around one or both eyes and nose in the 300 and 1000 mg/kg/day males and females. During the handling observations of the FOB at Weeks 10 (prior to dosing) and 11 (after dose removal), unkempt appearance (slightly soiled to very soiled fur) was noted in the 1000 mg/kg/day males and females. During Week 11 (after dose removal), total motor activity was decreased ($p<0.05$) in *all* treated male groups during the 0-10 and 11-20 minute intervals and *all* treated female groups during the 11-20 minute interval. These decreases in the males resulted in decreases (not dose-dependent, $p<0.05$) in cumulative total activity of 26, 40, and 26% in the 100, 300, and 1000 mg/kg/day groups, respectively. The motor activity decrease is consistent with the decrease seen in the oral acute neurotoxicity study noted following dosing. No compound-related effects were observed in mortality, body weight, body weight gain, food consumption, ophthalmoscopic exams, hematology, clinical chemistry, absolute or relative organ weights, or gross or microscopic pathology in either sex. Application site dermal effects were limited to very slight to slight erythema noted in the 300 and 1000 mg/kg/day groups and are considered self limiting.

The NOAEL (systemic) < 100 mg/kg/day. The LOAEL is 100 mg/kg/day based on decreased motor activity in both sexes at all dose levels tested.

The LOAEL (local irritation) = 300 mg/kg/day based on erythema. The NOAEL is 100 mg/kg/day.

This study is classified as acceptable/guideline and satisfies the guideline requirement for a 90-day dermal toxicity study (OPPTS 870.3250; OECD 411) in rats.

A.4.2 Prenatal Developmental Toxicity

870.3700a Prenatal Developmental Toxicity Study – Rat

In a developmental toxicity study (2008, MRID 47755402), Demiditraz technical (PF-3814927; 100% a.i.; Batch # PFXU070002) in 0.5% methylcellulose and 0.1% Tween^{TM} 80 was administered daily via gavage in a dose volume of 10 mL/kg to 25 time-mated presumed pregnant Sprague Dawley rats/dose group at dose levels of 0, 5, 25, or 100 mg/kg/day from gestation days (GD) 6-19. Blood samples were collected from 4 randomly selected rats per group 1 hour after dose administration on GD 19 to determine test substance levels in plasma. On GD 29, all maternal rats were euthanized; each dam's uterus and ovaries were removed via cesarean section and the contents examined. The fetuses were examined for external, visceral, and skeletal malformations and variations.

Plasma concentrations. Demiditraz concentrations in plasma increased in a dose-dependent manner, with measurements of 2.41, 39.9, and 1020 ng/mL in the 5, 25, and 100 mg/kg/day groups.

Maternal toxicity. At 100 mg/kg/day, one female (# 83309) was found dead approximately 0.5 hours following dose administration on GD 19. At the 0.5 hour post-dose observations, this female had: behavioral findings of subdued appearance upon cage-side observations; rocking, lurching, or swaying while ambulating on 3 to 5 occasions during GD 6-18; and a single occurrence of clear material around the mouth on GD 14. Although a cause of death could not be determined at necropsy, this death was considered treatment-related because it occurred around the time of expected peak exposure (0.5 hours post-dose), and the indications of maternal toxicity (post-dosing clinical observations, decreased body weight gain, and decreased food consumption) were similar to the findings found in the rest of the animals in this group. All other dams survived until scheduled termination.

At 100 mg/kg/day, the dams exhibited numerous clinical signs of toxicity at the 0.5-hour post-dosing examinations including rocking, lurching, or swaying while walking, piloerection, dilated pupils, subdued appearance upon cage-side observation only (sitting with head held low and appeared hypoactive but exhibiting normal behavior when stimulated), and wet clear material around mouth. These findings were noted as early as the first day of dose administration (GD 6) and generally continued throughout the treatment period. Additionally, salivation immediately prior to dose administration was noted at this dose but was attributed to a response to the taste or irritating properties of the test material and was not considered adverse.

At 100 mg/kg/day, body weights were decreased by 5-6% ($p<0.05$) on GD 18, 19, and 20. Boy weight gains were decreased ($p<0.01$) by 58% during GD 6-10, by 16% for GD 14-20, and by 20% for the overall (GD 6-20) treatment period. Gravid uterine weights were decreased by 12% ($p<0.01$), and corrected body weight gain for the overall (GD 0-20) study were decreased by 15% ($p<0.05$). Absolute and relative food consumption was decreased ($p<0.01$) by 14-15% for GD 6-10 and by 6-11% for GD 6-20. Additionally at this dose, absolute food consumption was decreased by 7% for GD 14-20.

The maternal LOAEL is 100 mg/kg/day based on mortality, clinical signs of toxicity, and on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 25 mg/kg/day.

Developmental toxicity. There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses. Furthermore, there were no treatment-related effects on the numbers of litters, early resorptions, late resorptions, or live fetuses. Sex ratio and post-implantation loss were unaffected by treatment.

Fetal *variations* were limited to the incidences of delayed ossification. At 100 mg/kg/day, fetal body weights were decreased by 11% ($p<0.05$) each in the males and females. The incidence of sternebra(e) #5 and/or #6 unossified was increased at 100 mg/kg/day (42.3% fetuses; 95.7% litters) compared to concurrent controls (26.1% fetuses; 87.0% litters) and historical controls (0.2-23.1% fetuses). Similarly, the incidence of reduced ossification of the vertebral arches was increased at 100 mg/kg/day (1.9% fetuses; 17.4% litters) compared to concurrent controls (0%) and historical controls (0.0-1.1% fetuses). Several other skeletal variations characterized by delayed ossification differed from concurrent controls at 100 mg/kg/day and, although the fetal incidences of these findings fell within the range of historical controls, they were considered to be treatment-related because they corresponded to the statistically significant, treatment-related decreases in fetal body weights at this dose. These variations included: (i) increases in sternebra(e) #1, 2, 3, and/or 4 unossified at 100 mg/kg/day (1.1% fetuses; 17.4% litters) compared to concurrent (0.3% fetuses; 4.3% litters) and historical (0.0-1.3% fetuses) controls; (ii) increases in pubis unossified at 100 mg/kg/day (1.6% fetuses; 4.3% litters) compared to concurrent (0%) and historical (0.0-2.3% fetuses) controls; (iii) increases in hyoid unossified at 100 mg/kg/day (2.3% fetuses; 17.4% litters) compared to concurrent (1.4% fetuses; 17.4% litters) and historical (0.0-4.2% fetuses) controls; and (iv) decreases in incidence of ossified cervical centrum #1 at 100 mg/kg/day (12.0% fetuses; 65.2% litters) compared to concurrent (19.7% fetuses; 78.3% litters) and historical (6.6-35.8% fetuses) controls.

There were no treatment-related external, visceral, or skeletal *malformations*.

The developmental LOAEL is 100 mg/kg/day based on decreased fetal body weights and associated delayed ossification of the skeleton. The developmental NOAEL is 25 mg/kg/day.

This study is classified acceptable/guideline and satisfies the guideline requirement (OPPTS 870.3700a; OECD 414) for a developmental toxicity study in rats.

870.3700b Prenatal Developmental Toxicity Study – Rabbit

In a developmental toxicity study (2008, MRID 47755401), Demiditraz technical (PF-3814927; 100% a.i.; Batch # PFXU070002) in 0.5% methylcellulose and 0.1% Tween^{TM} 80 was administered via gavage in a dose volume of 5 mL/kg to 25 time-mated presumed pregnant New Zealand White rabbits/dose group daily from gestation days (GD) 6-28. Initial doses were 0, 10, 50, and 200 mg/kg/day. Due to excess toxicity, the high dose level was lowered to 150 mg/kg/day on GD 9, 10, or 11 for the remainder of the study. Blood samples were collected from 4 rabbits per group 1 hour after dose administration on GD 28 to determine test substance

levels in plasma. On GD 29, all maternal rabbits were euthanized; each doe's uterus and ovaries were removed via cesarean section and the contents examined. The fetuses were examined for external, visceral, and skeletal malformations and variations.

Plasma concentrations. Demiditraz concentrations increased in a dose-dependent manner, with measurements of 411, 1940, and 5390 ng/mL in the 10, 50, and 200/150 mg/kg/day animals. It was stated that this time point was the expected maximum plasma concentration (0.5 to 2.2 hours) based on the previously conducted range-finding study (Bowman, 2007, WIL-344041).

Maternal toxicity. The morbidity in the three animals at the high dose occurred early in the study (GD 6 or 9) when the animals were receiving 200 mg/kg/day. The female that was euthanized *in extremis* at 50 mg/kg/day was observed with tremors and rales approximately 30 minutes following dose administration on GD 14, two days prior to euthanasia on GD 16. The deaths/euthanasia in the 50 and 200/150 mg/kg/day groups occurred along with clinical signs indicating neurotoxicity (tremors, prostration, and/or clonic convulsions) approximately 30 minutes following dose administration.

At 50 mg/kg/day, body weights were decreased by 6% ($p<0.05$) on GD 14-16. At 200/150 mg/kg/day, body weights were decreased by 5-9% beginning on GD 8 and continuing throughout the remainder of the study; with the exception on GD 26 and 27, these decreases were statistically significant ($p<0.05$). Statistically significant ($p<0.05$) body weight losses were noted in the 50 mg/kg/day (-11 g) and 200 mg/kg/day (-154 g) groups compared to a body weight gain in the control group of 35 g at the beginning of the dose administration period (GD 6-9). For GD 9-12, body weight gains were decreased by 88% ($p<0.01$), and a significant ($p<0.01$) body weight loss was observed at 200/150 mg/kg/day (-4 g) compared to a body weight gain in controls (58 g). For the overall treatment period (GD 6-29), a significant ($p<0.01$) body weight loss was noted at 200/150 mg/kg/day (-51 g) compared to a body weight gain in the controls (215 g).

Absolute and relative (to body weight) food consumption was decreased ($p<0.01$) by 20-27% at 50 mg/kg/day for GD 6-9 and 9-12 and by 42-65% at 200/150 mg/kg/day for GD 6-9, 9-12, and 12-21. Additionally at 50 mg/kg/day, absolute food consumption was decreased by 24% ($p<0.05$) for GD 12-21. Absolute and relative food consumption for the overall (GD 6-29) treatment period were decreased ($p<0.05$) by 15-20% at 50 mg/kg/day and by 38-42% at 200/150 mg/kg/day. No treatment-related findings were found at necropsy. The maternal LOAEL is 50 mg/kg/day based on incidences of mortality/moribundity, clinical signs of toxicity, and decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 10 mg/kg/day.

Developmental toxicity. There were no complete litter resorptions or dead fetuses. Furthermore, there were no treatment-related effects on the numbers of early resorptions, late resorptions, or live fetuses per doe. Sex ratio was unaffected by treatment. There were no treatment-related external, visceral, or skeletal *malformations*.

At the high dose, fetal body weights were decreased by 12% (not significant [NS]) in the males and by 13% ($p<0.05$) in the females. Fetal and litter incidences of rabbits with 27 presacral

vertebrae were dose-dependently increased at 50 mg/kg/day (15.0% fetuses, 54.5% litters) and 200/150 mg/kg/day (29.6% fetuses, 64.7% litters) compared to concurrent controls (5.0% fetuses, 26.1% litters). The fetal incidences of this variation at 50 mg/kg/day fell within the range of historical controls (4.8-15.6% fetuses); however, the incidence at 200/150 mg/kg/day exceeded the range of historical controls. Therefore, the incidences of 27 presacral vertebrae at 200/150 mg/kg/day were considered to be due to treatment. Similarly, the fetal incidences of rabbits with full 13th rib(s) were dose-dependently increased at 50 mg/kg/day (42.9% fetuses, 90.9% litters) and 200/150 mg/kg/day (57.2% fetuses, 88.2% litters) compared to concurrent controls (33.2% fetuses, 91.3% litters). The fetal incidences of this variation at 50 mg/kg/day fell within the range of historical controls (16.5-45.7% fetuses); however, the incidence at 200/150 mg/kg/day exceeded the range of historical controls. Therefore, the incidences of full 13th rib(s) at 200/150 mg/kg/day were considered to be due to treatment. However, it should be noted that these findings (27 presacral vertebrae and 13th full ribs) comprise two of the three most commonly observed skeletal variations. The developmental LOAEL is 200/150 mg/kg/day based on decreased fetal body weights and slight increases in the incidences of 27 presacral vertebrae and 13th full rib(s). The developmental NOAEL is 50 mg/kg/day. Note: Apparent increases in the skeletal variations at the high dose were also evident in the 50 mg/kg/day dose but were within historical control range.

This study is classified acceptable/guideline and satisfies the guideline requirement (OPPTS 870.3700b; OECD 414) for a developmental toxicity study in rabbits.

A.4.3 Reproductive Toxicity

870.3800 Reproduction and Fertility Effects – Rat

In a two-generation reproduction toxicity study (2009, MRID 47744117), Demiditraz (PF-03814927; 100% a.i.; Batch #PFXU070002) in 0.5% methylcellose and 0.1% Tween® 80 (10 mL/kg) was administered daily by gavage at dose levels of 0, 7.5, 50, or 150 mg/kg/day for two consecutive generations. The P generation animals (30/sex/dose group) were dosed for 10 weeks prior to mating to produce the F1 litters. F1 offspring (30/sex/dose group) were dosed after weaning for at least 10 weeks prior to mating to produce the F2 generation.

In the P and F1 generations, treatment-related generally transient clinical findings indicative of neurotoxicity were noted with increased incidences in the 50 and 150 mg/kg/day group at approximately 0.5 hours following dose administration starting as early as study day 0 and lasting through study day 115. The findings were numerous and included effects such as subdued appearance; rocks, lurches, or sways as it walks; pupils dilated; and hypoactivity. Additionally at 150 mg/kg/day, increased incidences tremors in both sexes and respiratory effects (except in F1 females) were noted and clonic convulsions were noted in females. Gasping was noted especially in F1 male pups at 150 mg/kg/day.

In the P generation, one 150 mg/kg/day female died, exhibiting clonic convulsions immediately following dosing. In the F1 generation following weaning and starting gavage of the pups, the numbers of decedents were higher in the 150 mg/kg/day group (7/sex) than the controls (2/sex). The majority of these deaths occurred during PND 23-26.

In the P generation, decreases were observed in body weights and gains on LD 7, 14, and 21 at 50 mg/kg/day and throughout the *lactation period* at 50 and 150 mg/kg/day. Decreased food consumption was noted at 150 mg/kg/day. Decreased food efficiency was observed at 50 and 150 mg/kg/day. In the F1 generation at 150 mg/kg/day, decreases were observed in body weights throughout the lactation period; however, the resulting decrease in body weight gain was not significant. Decreased food consumption was noted in the 150 mg/kg/day group.

At 150 mg/kg/day, decreased body weights and gains were noted in the P and F1 generations males. Average food efficiency (Days 0-126) was decreased in these males. Average food efficiency (PND 28-161) was also decreased. Decreased body weights were noted in the 150 mg/kg/day females in the F1 generation up to PND 91 resulting in a decreased body weight gain for the pre-mating period.

At 150 mg/kg/day, decreases in body weights throughout the *gestation period* in the P and F1 generations, resulting in a decreased overall (GD 0-20) body weight gain. Food efficiency was decreased during gestation in both generations.

No adverse, treatment-related effects were noted on organ weights or gross or histological pathology in either generation.

The LOAEL for parental toxicity is 50 mg/kg/day based on: clinical findings indicating nervous system toxicity in both sexes and generations; and decreased body weight, body weight gain, and food efficiency during *the lactation period* in the P generation. The NOAEL is 7.5 mg/kg/day.

At 150 mg/kg/day, decreases were noted in the number of pups born (↓13% and 16%) and born alive (both ↓18%) in both the F1 and F2 generations. There was also an increase in the number of implantation sites that did not result in the birth of a pup in both the F1 and F2 litters. The viability indices were decreased in the F1 (↓19%) and F2 (↓29%) generations. The lactation indices in the treated groups of both generations were similar to controls. Decreased postnatal survival (% per litter) was observed during PND 0-1 in the F1 and F2 generations and during PND 1-4 in the F2 generation.

At 50 mg/kg/day in the F2 generation, decreased body weights were noted in both sexes (↓8-11% in males and 8-12% in females) throughout lactation, and decreased body weight gains (up to 12%) were observed in the males at PND 4-7, 7-14, and 14-21 and females at PND 4-7 and 7-14. At 150 mg/kg/day, decreased pup body weights (up to 31%) were observed during PND 1-21 in both sexes and generations. Except for the F1 males and females on PND 1-4, body weight gains during PND 1-21 were also decreased in both sexes and generations (up to 45%).

Absolute brain weights were decreased in the F2 generation at 50 mg/kg/day (↓4-6%) in both sexes in the F2 generation. At 150 mg/kg/day absolute brain weight was decreased 6-13%.

No treatment-related macroscopic findings were observed in the F1 and F2 generations. Pup sex ratio and time until vaginal opening or balano-preputial separation were unaffected by treatment. Developmental landmarks were not reported.

The LOAEL for offspring toxicity is 50 mg/kg/day based on decreased pup body weights and body weight gains in both sexes in the F2 generation and slight decrease in brain weight in the F2 generation. The NOAEL is 7.5 mg/kg/day.

There were no effects of treatment in either generation on: estrous cycle; sperm parameters; mating, conception, fertility, or gestation indices; pre-coital interval; or gestation duration in either generation.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 150 mg/kg/day.

This study is classified as acceptable/guideline and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

A.4.4 Chronic Toxicity – No study available

A.4.5 Carcinogenicity – No study available

A.4.6 Mutagenicity

Demiditraz was negative in the mutagenicity studies.

Study	Dose Levels	Result
***In vitro* studies**		
870.5100 **Bacterial Reverse Mutation** MRID#47752704 Mecchi, M.S. (2008)	0-5000 µg/plate	Negative
870.5300 **Mammalian cell gene mutation (CHO)** MRID #47755404 Stankowski, L.F. (2008)	0-900 µg/mL	Negative
870.5375 **Mammalian cytogenetics assay (chromosomal aberration assay in human peripheral blood lymphocytes)** MRID #47752705 Murli, H. (2008)	0-2000 µg/mL	Negative
***In vivo* studies**		
870.5395 **Mouse Bone Marrow Micronucleus Assay** MRID#47755403 Xu, Y (2008)	0-1000 mg/kg bw	Negative

A.4.7 Neurotoxicity

870.6200a Acute Neurotoxicity Screening Battery – Acute Rat

In an acute neurotoxicity screening study (2008, MRID 47744113), PF-3814927 (Demiditraz, 99.2% a.i., Batch # PFXU060001) in 0.5% methylcellulose/0.1% polysorbate 80 was administered once via gavage (5 mL/kg) to 12 Sprague-Dawley rats/sex/group at dose levels of 0, 15, 100, or 600 mg/kg. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on 12 rats/sex/group at pre-dosing and days 0 (approximately 15 minutes post-dosing; time of peak effect), 7, and 14. At study termination, 6 rats/sex/group were perfused *in situ* for neuropathological examination. The tissues from the perfused animals in the control and 600 mg/kg groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

Motor Activity. Findings at 15 mg/kg were limited to *decreased* ($p<0.05$) mean total and ambulatory activity counts in the females (decr 17-18%) during the 0-15 minute interval on Day 0. Mean total and ambulatory activity counts were *decreased* ($p<0.001$) by 90-96% in males and 88-96% in females at 100 mg/kg and 600 mg/kg. At the higher doses, these decreases resulted in decreases ($p<0.001$) in cumulative total (decr 68-90%) and ambulatory (decr 74-93%) activity counts. No statistically significant differences in motor activity were noted on Days 7 or 14. Although these values for the low dose group were lower than the minimum historical control values provided, these decreases were not sufficient to affect cumulative total or ambulatory activity counts, as animals at this dose had higher (not statistically significant) values than the concurrent control group for the remainder of the 60-minute test session.

FOB evaluations on Day 0. At 100 and 600 mg/kg, numerous effects were noted but these findings were generally resolved by Day 7. During the home-cage observations, the following treatment-related effects (# affected/12 vs. 0/12 controls, unless otherwise noted) were reported: (i) *flattened posture* (9 males and 11 females at 100 mg/kg, and 10 males and 7 females at 600 mg/kg); (ii*) immobility* (9 males and 7 females at 100 mg/kg, and 7 males and 4 females at 600 mg/kg); and (iii) *eyelids slightly drooping* (6 females at 100 mg/kg, and 6 males and 7 females at 600 mg/kg vs. 3 control males and 0 control females). Additionally at 600 mg/kg on Day 0, *slight tremors* were observed in one male and 3 females, and moderately *coarse tremors* were observed in 2 males. *Clonic convulsions* (whole body tremors) were also observed at 600 mg/kg in 2 males and 1 female.

During handling observations, eyelids slightly drooping were noted in 7 rats of each sex at 100 mg/kg and in 10 males and 6 females at 600 mg/kg (vs. 0 controls). Additionally at 600 mg/kg, 6 males displayed slight *resistance to handling* (vs. 1 control). During the sensory observations, increased numbers of animals displayed no reaction to the following stimuli: (i) approach; (ii) touch; (iii) tail pinch; and (iv) startle. Several animals in these groups also exhibited no olfactory response, were slightly uncoordinated (3-6 males and 2-9 females) or landed on their sides (4 males and 1 female at 600 mg/kg only) during air-righting reflex assessment. Additional findings, occurring in 1 or 2 animals at 600 mg/kg, consisted of no eye blink response, no pupil response and no forelimb extension.

During the open-field observations, the following treatment-related effects were noted: (i) impaired mobility, slight (5 males and 6 females at 100 mg/kg, and 7 males and 11 females at 600 mg/kg), moderate (3 males at 600 mg/kg), and totally impaired (1 rat of each sex at 600 mg/kg); (ii) abnormal gait, body drags (5 males and 2 females at 600 mg/kg), hindlimbs splayed or dragging (6 males and 5 females at 600 mg/kg), hunched body (4 males and 3 females at 100 mg/kg, and 2 males and 1 female at 600 mg/kg), and ataxia (8 females at 100 mg/kg, and 7 males and 10 females at 600 mg/kg); (iii) gait score, slight impairment (6 rats each sex at 100 mg/kg, and 4 rats each sex at 600 mg/kg), considerable impairment (1 rat each sex at 100 mg/kg, and 5 males and 6 females at 600 mg/kg), severe impairment (1 male), and marked impairment (1 female) at 600 mg/kg); (iv) tremors, slight (2 males and 3 females at 600 mg/kg), moderately coarse (2 males and 1 female at 600 mg/kg), and markedly coarse (one 600 mg/kg female); and (v) arousal, low (6 of each sex at 100 mg/kg, and 9 of each sex at 600 mg/kg vs. 1 control female), and very low (one 600 mg/kg male). Additionally, one 600 mg/kg male displayed circling and retropulsion. Rearing counts were decreased ($p<0.01$) in the males (0.6 to 1.8 treated vs. 5.8 controls) and females (1.5 to 2.9 treated vs. 7.6 controls). Rearing counts in these males remained decreased on Day 7; however, the differences were not statistically significant. Urination counts were increased ($p<0.05$) in the ≥100 mg/kg males (1.4 to 1.7 treated vs. 0.2 controls) and females (1.2 to 1.7 treated vs. 0.2 controls).

During the neuromuscular observations on Day 0, treatment-related decreases ($p<0.01$) in hindlimb footsplay (decr 31-48%) and rotarod performance (decr. 55-67%) were noted at 600 mg/kg in both sexes. During physiological observations on Day 0, body temperatures (°C) were decreased ($p<0.01$) at 100 and 600 mg/kg in both sexes (35.1 and 34.5 treated, respectively, in males vs. 37.2 controls, and 35.2 and 34.6 treated, respectively, in females vs. 37.8 controls).

No compound-related effects were observed in mortality, body weight (except of initial decrease in day 0-7 gain at 600 mg/kg), brain weight and morphology, or gross and neuropathology in either sex.

The LOAEL was 100 mg/kg based on FOB findings (predominantly altered gait and posture, impaired mobility, decreased rearing, incoordination, increased urination, and lower body temperature) and statistically significant decreases in motor activity in both sexes. The NOAEL is 15 mg/kg. The slight decrease in interval motor activity at 15 mg/kg is a possible threshold effect.

The study is classified as acceptable/guideline and satisfies the guideline requirement (OPPTS 870.6200a) for an acute neurotoxicity study in rats.

870.6200b Subchronic Neurotoxicity Screening Battery – Rat

In a subchronic neurotoxicity study (2008, MRID 47744114), PF-3814927 (Demiditraz, 100% a.i.; Batch No. PFXU070002) in 0.5% methylcellulose and 0.1% Tween 80® was administered daily via gavage (5 mL/kg) to 22 Sprague-Dawley rats/sex/group at dose levels of 0, 5, 25, or 125 mg/kg/day for 13 weeks. Ten rats/sex/dose were designated as the Phase I (general toxicity) group, and were evaluated for hematology, clinical chemistry, ophthalmology, organ weight, and gross and histopathology parameters. Neurobehavioral assessment (functional observational

battery [FOB] and motor activity testing) was performed on the remaining 12 rats/sex/group (Phase II) at pre-dosing and Weeks 1, 3, 7, and 12 (prior to administration of dosing). At study termination, all Phase II animals were anesthetized and perfused *in situ* for brain weights and morphometry. The tissues from 6 rats/sex/group of the perfused animals in the control and 125 mg/kg/day groups were subjected to histopathological evaluation of central and peripheral nervous system tissues.

At 25 mg/kg/day, body weights were decreased ($p<0.05$) by 7-8% in the males during Weeks 8-13 and by 7% each in the females at Weeks 7 and 10. Body weight gains were decreased ($p<0.05$) by 33% in the males during Weeks 7-8. Overall body weight gains were decreased ($p<0.05$) by 11% in the males. At 30 minutes post-dosing, the following treatment-related clinical signs of toxicity were observed (# affected/22 vs. 0/22 controls, unless otherwise stated): (i) subdued appearance at cage-side observation only (10 males and 9 females); (ii) rocks, lurches, or sways when walking (6 males and 6 females); (iii) hunched posture (6 females); (iv) hypoactivity (2 males); (v) wet yellow material urogenital area (3 males and 6 females); (vi) lacrimation right/left eye (8 males and 19 females vs. 2 control females); (vii) dilated pupil right/left eye (5 males and 6 females); (viii) dried red material around mouth (4 males and 1 female vs. 1 control male); and (ix) wet red material around mouth (2 males). Cumulative ambulatory activity was *increased* ($p<0.05$) by 41-45% in the males at Weeks 3 and 7.

At 125 mg/kg/day, body weights were decreased ($p<0.01$) by 9-14% in the males and by 7-10% in the females during Weeks 3-13. Additionally, body weight gains were sporadically decreased ($p<0.05$) by 14-48% in the males and by 17-56% in the females. Overall (Weeks 0-13) body weight gains were decreased ($p<0.01$) by 15-19% in both sexes. At 30 minutes post-dosing, the following treatment-related clinical signs of toxicity were observed (# affected/22 vs. 0/22 controls, unless otherwise stated): (i) subdued appearance at cage-side observation only (all males and females); (ii) rocks, lurches, or sways when walking (all males and females); (iii) hunched posture (17 males and all females); (iv) hypoactivity (11 males and 3 females); (v) wet yellow material urogenital area (20 males and 21 females); (vi) shallow respiration (7 males and 1 female); (vii) decreased respiration (10 males); (viii) lacrimation right/left eye (20 males and all females vs. 2 control females); (ix) dilated pupil right/left eye (20 males and 19 females); (x) dried red material around mouth (9 males and 11 females vs. 1 control male); and (xi) wet red material around mouth (8 of each sex). Cumulative (session) total activity was *increased* by 29-32% in the males at Weeks 1 and 3 and by 31-32% in the females throughout the study. Also at this dose, cumulative ambulatory activity was increased by 43-56% in the males at Weeks 1, 3, and 7 and by 49-65% in the females throughout the study.

No compound-related effects were observed in mortality, food consumption, ophthalmoscopic examinations, hematology, clinical chemistry, FOB parameters (note: FOB assessments were made prior to daily dosing), brain weight and morphology, organ weights, gross lesions, or microscopic lesion in either sex.

The LOAEL was 25 mg/kg/day based on multiple clinical signs of toxicity in both sexes, decreased body weight gains in males, and increased motor activity in males. The NOAEL is 5 mg/kg/day.

The study is classified as acceptable/guideline and satisfies the guideline requirement (OPPTS 870.6200b; OECD 424) for a subchronic neurotoxicity study in rats.

870.6300 Developmental Neurotoxicity Study

In a developmental neurotoxicity study (MRID 48766703), demiditraz (PF-03814927) (100% purity; Batch 1 Lot # TCK08005K and Batch 2 Lot # TCK08005K) was administered daily by oral gavage to 25 mated female Sprague-Dawley [Crl:CD(SD)] rats per dose at dose levels of 0 (0.5% methylcellulose (400 cps)/0.1% Tween® 80 in deionized water), 5, 15, or 100 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 20.

Detailed clinical observations were conducted on all dams (F_0 females) on GD 10 and GD 15 and on LD 10 and LD 20. All F_0 females were allowed to deliver and rear their offspring to LD 21. All surviving F_0 females were euthanized on LD 21 and subjected to a gross necropsy. Clinical observations, body weights, and sexes were recorded for the F_1 pups at appropriate intervals. On postnatal day (PND) 4, litters were culled to 8 pups/litter, and a subset (Subset A) of 20 pups/sex/group was assigned to the functional observational battery (FOB; PND 4, 11, 21, 35, 45, and 60), auditory startle response (PND 20 and 60), locomotor activity (PND 13, 17, 21, and 61), and learning and memory (PND 62) groups. From this subset, 15 pups/sex/group were deeply anesthetized and perfused *in situ* on PND 72 for brain weight and brain measurement evaluations; of these, 10 pups/sex/group were selected for neuropathological and brain morphometric evaluations. A second subset (Subset B) of 20 pups/sex/group was selected for learning and memory (PND 22). A third subset (Subset C) of 15 pups/sex/group was deeply anesthetized and perfused *in situ* on PND 21 for brain weight and brain measurement evaluations; of these, 10 pups/sex/group were selected for neuropathological and brain morphometric evaluations. Indicators of physical development (balanopreputial separation and vaginal patency) were evaluated for all F_1 selected pups in Subsets A and B. All F_1 pups not selected for neuropathological or behavioral evaluations were euthanized and necropsied on PND 21. F_1 pups selected for learning and memory assessment on PND 22 were necropsied following attainment of sexual developmental landmarks. The litter was used as the experimental unit for all F_1 data.

One F_0 female in the 100 mg/kg/day group was found dead on GD 21, and cause of death could not be determined. All other F_0 females survived to the scheduled euthanasia. One female each in the control and 100 mg/kg/day groups failed to deliver and were determined to be non-gravid. Treatment-related clinical findings were observed in a dose-dependent manner in the 15 and 100 mg/kg/day group F_0 females and were observed at approximately 15-30 minutes post-dosing throughout the treatment period. In both dose groups, these findings included sitting with the head held low, hypoactivity, a flattened body, slightly drooping eyelids, decreased respiration, clear material around the mouth/salivation, lacrimation, and dilated pupils. Additional signs seen in the 100 mg/kg/day dose group included rocking, lurching, or swaying while ambulating, piloerection, tremors/convulsions, and yellow material on various body surfaces. These findings generally continued to the 2-hour post-dosing observation period for the 100 mg/kg/day group F_0 females, but at decreased incidences.

Treatment-related decrements in maternal care were observed mainly in the 100 mg/kg/day group F_0 females during the same interval post dose when clinical signs were observed. A decreased incidence of F_0 females on the nest, a higher incidence of females that were away from the nest (not actively eating, drinking, grooming or tending to the litter), and higher incidences of scattered litters with 1-3 pups and more than 3 pups outside of the nest were all noted at the 100 mg/kg/day dose level.

During the detailed clinical observations (FOB) for F_0 females, a flattened posture or sitting with the head held low, low or very low arousal, slight to moderately coarse tremors, lacrimation, salivation, piloerection, slightly drooping eyelids, decreased respiration, soft and flabby muscle tone, impaired mobility, ataxia, dragging bodies, and/or higher urination counts were observed for the 100 mg/kg/day group at each time points. Additionally, a flattened posture or sitting with the head held low, ataxia, impaired mobility, and a higher urination count were observed in a smaller number of F_0 females at 15 mg/kg/day from GD 15 on.

Mean body weights for F_0 females in the 100 mg/kg/day group were slightly (↓4.6%) lower than the control group by GD 20. F_0 females in the 100 mg/kg/day group had slightly lower mean body weight gains throughout the gestation treatment period (initially ↓44%; overall GDs 6-20, ↓15%), and corresponding reductions in food consumption during GDs 6-12 (↓13-14). Mean body weights for F_0 females in the 100 mg/kg/day group were slightly (↓4% to 6%) lower than the control group during LDs 4-17, but were similar to the control group on LD 21. A lower mean body weight gain was noted for F_0 females in the 100 mg/kg/day group during LDs 1-4 (67% less than controls), but mean body weight gains for this group were generally similar to the control group for the remainder of the lactation treatment period (LDs 4-21). Mean gestation length of F_0 females was comparable among the groups, and there were no treatment-related effects on the process of parturition or macroscopic findings of the F_0 females.

The maternal LOAEL is 15 mg/kg/day, based on clinical signs (sitting with the head held low, hypoactivity, a flattened body, slightly drooping eyelids, decreased respiration, clear material around the mouth/salivation, lacrimation, and/or dilated pupils). The maternal NOAEL is 5 mg/kg/day. At 100 mg/kg/day, in addition to clinical signs, decrements in maternal care were observed during the same time interval post dose when clinical signs were observed (decreased incidence of F_0 females on the nest, a higher incidence that were away from the nest (not actively eating, drinking, grooming or tending to the litter), and higher incidences of scattered litters with 1-3 pups and more than 3 pups outside of the nest.

The mean live litter size in the 100 mg/kg/day group was slightly lower than the control group (14.0 vs. 15.1 for controls), and mean postnatal survival for the 100 mg/kg/day group was lower than the control group during PND 0-1, 1-4 (pre-culling), and birth to PND 4 (pre-culling) (87.7% vs. 96.2% for the latter). At the 100 mg/kg/day dose level, a total of 24 pups were found dead and 20 others were missing (presumed cannibalized) compared with 11 found dead and 3 missing at the control level.

At 100 mg/kg/day, F_1 pups of both sexes had decreased body weights [PND 1 (↓8%-9%); PND 4 (↓14%); PND 21 ↓16%)] and body weight gains (PND 1-4 (↓21%-29%); PND 4-7 (↓27%); PND 1-21 (↓16%-18%) generally throughout the pre-weaning period, relative to their respective

controls. Mean body weights of the F_1 males at 100 mg/kg/day were significantly lower than the control (PND 28-72 ↓10%-14%), and lower mean body weight gains were noted for the 100 mg/kg/day F_1 males compared to the control throughout the post-weaning period (overall PND 28-72, ↓11%). Mean body weights for the 100 mg/kg/day F_1 females were lower than the control during PND 28-72 (↓5%-13%), and lower mean body weight gain was noted for the 100 mg/kg/day group F_1 females during the first post-weaning week (PND 28-35, ↓8%), but mean body weight gains were similar to the control for the remainder of the post-weaning period and overall.

A treatment-related delay (2.7 days) in the mean age of attainment of balanopreputial separation was noted for the 100 mg/kg/day group F_1 males (47.0 days vs. 44.3 days for controls) but mean body weights of the F_1 males on the day of attainment were not affected. In contrast the F_1 females showed no treatment related effects on the mean age of attainment of vaginal patency, but a reduced mean body weight (↓11%) on the day of attainment of vaginal patency was noted for the F_1 females of the 100 mg/kg/day group.

Mean forelimb grip strength for the 100 mg/kg/day group F_1 males and F1 females was significantly lower than the control group on PND 21 and PND 35 but was no longer significantly affected on PND 45 or PND 60.

Although no statistically significant effects on locomotor activity or habituation were observed among the F_1 offspring, there were differences in response between the control and 100 mg/kg/day groups and between the sexes at 100 mg/kg/day. On PND 13, the concurrent control males displayed motor activity (MA) that was not consistent with the testing facility's historical control data, which resulted in a finding of decreased MA at all dose levels (males). However, when compared to the historical control (HC) mean, the PND 13 males at 100 mg/kg/day showed an increase in MA (ambulatory ↑100%/total ↑45%) and the 5 and 15 mg/kg/day PND 13 males were comparable to the HC mean. PND 13 females at 100 mg/kg/day showed a reduction in MA (ambulatory ↓41%/total 20%) compared to the concurrent control. On PND 17, the 100 mg/kg/day males displayed reduced MA and the 100 mg/kg/day females displayed MA that was comparable to the control. On PND 21, MA among the male groups was comparable, whereas the 100 mg/kg/day females displayed an increase (ambulatory ↑40%/total ↑2%) compared to the control females. The male control and 5 and 15 mg/kg/day male dose groups showed the expected reduction in MA on PND 21 compared to PND 17 levels, but the 100 mg/kg/day males did not display the expected reduction in MA. On PND 61, males at 100 mg/kg/day displayed a reduced activity (ambulatory ↓18%/total ↓14%), and females at 100 mg/kg/day were comparable to the control.

Both sexes at 100 mg/kg/day displayed an increase in auditory startle response peak amplitude on PND 60 compared to the concurrent controls and the historical controls. The PND 60 males at 100 mg/kg/day had a mean peak amplitude value that was outside the HC, whereas the PND 60 females at 100 mg/kg/day were within the HC range, although at the upper end. Additionally, both sexes at 100 mg/kg/day displayed a reduction of 4 msec in latency time on PND 60 compared to the concurrent control and HC control.

At 100 mg/kg/day, mean brain weight was significantly lower in PND 21 males compared to the control, and relative brain weights were significantly increased in both sexes at 100 mg/kg/day on PND 21. Relative brain weight was significantly lower in the PND 60 males at 100 mg/kg/day.

No treatment related effects were observed in the detailed clinical observations performed on PNDs 4, 11, 21, 35, 45, or 60 during the home cage, handling, open field, or sensory assessments. There were no treatment-related effects on the numbers of former implantation sites or unaccounted-for sites, the pup sex ratio at birth, pup survival following culling, clinical findings, swimming ability or learning and memory assessments, macroscopic internal findings, gross or microscopic observations of the brain or peripheral nervous systems, brain measurements at necropsy, or morphometry taken from brain sections.

The F_1 offspring LOAEL is 100 mg/kg/day, based on decreased postnatal survival, decreased pup body weights and body weight gains, increased maximum response to the auditory startle stimulus and reduction in response time on PND 60, decreased/increased total and ambulatory motor activity counts, decreased grip strength (PND 21 and PND 35), decreased brain weight in PND 21 males, and delayed attainment of balanopreputial separation. The corresponding F_1 offspring NOAEL is 15 mg/kg/day.

This study is classified Acceptable/Guideline and satisfies the guideline requirement for a developmental neurotoxicity study in rats [OCSPP 870.6300 (§83-6); OECD 426 (draft)].

A.4.8 Metabolism

870.7485 Metabolism - Rat

No guideline study is available.

870.7600 Dermal Absorption – Rat

In a dermal penetration study (2008, MRID 47752709), [^{14}C] PF-03814927 (Demiditraz; 99.0% radiochemical purity; Code No. CFQ15145 Batch 1) formulated in diethylene glycol monobutyl ether was applied to the shaved skin (10 cm^2) of Sprague Dawley rats. Doses of 0.0099, 0.0937, or 1.03 mg/rat were applied to the skin of each rat in volumes of 10 µl/cm^2 skin. Four males/dose/time point were tested using exposure durations and termination times of 0.5, 1, 2, 4, 10, or 24 hours post-administration. Additional groups of 4 male rats was exposed for 10 hours at the same dose levels, the application site was washed, and the rats were terminated at 168 after application.

Analytical recoveries were 76-108% of the applied dose (AD). The amount absorbed included residues found in the cage wash, blood, urine, feces, and carcass. After 24 hours exposure, there was 17.46%, 12.06% and 10.61% of the AD was determined to be absorbed for the low , mid an high dose exposure groups, respectively. The groups terminated at 168 hours post-application indicated additional absorption (25.8-28.1% AD) resulting from absorption from the skin depots

(skin at the application site and adjacent skin). The absorption rate constants were similar (0.055-0.089/hr) for each dose level. Thus, absorption was not saturated.

Because absorption continued to occur from demiditraz remaining at the site of application after washing, the total absorbable dose (AD plus remaining on the skin) should also be considered in a conservative estimate of absorption. The mid-dose provided the most conservative estimate of total absorbed (28.06%) and absorbable (3.09%) for a total of 31% and should be considered for a dermal penetration factor in risk assessment.

Quantifiable residues were detected in the urine and feces by 2 and 10 hours after application, respectively. Only minor amounts were found in the blood and cage wash. The carcass accounted for ~1-8% AD with the highest percentages in the low dose group. The majority of the residues eliminated in the feces and urine were excreted within 72 hours. Regardless of dose level, the elimination rate constants were similar in both the urine and feces (0.0227-0.0291/hr). The elimination half life was 28-31 hr in urine and 24-28 hr in feces.

A conservative estimate of dermal absorption is 31% based on total absorbed and remaining on the skin after 168 hours.

This study is classified as acceptable/guideline and satisfies the guideline requirements (OPPTS 870.7600; OECD - none) for a dermal penetration study in rats. The study provides data that can be useful to derive a dermal absorption factor. It must be noted that the test material was applied in diethylene glycol monobutyl ether and the relationship of this vehicle to practical formulations in not known.

A.4.9 Immunotoxicity

870.7800 Immunotoxicity

In an immunotoxicity study (MRID 47744122), female Sprague Dawley Crl:CD(SD) rats (20/dose group) was administered daily via oral gavage with demiditraz (PF-3814927; 99.5% a.i.; Batch # PFXA070001) in 0.5% methylcellulose and 0.1% Tween^{TM} 80 at dose levels of 0, 10, 30, or 100 mg/kg/day with a dose volume of 10 mL/kg for 28 days. Splenic antibody-forming cell (AFC) assays were conducted on 10 rats/dose group (AFC Group), and the remaining 10 rats/dose group were used for Natural Killer (NK) cell activity assay, immune cell phenotyping, and anti-CD3 stimulation assay (non-AFC group). In addition, two positive control groups (10 rats/group) were included. For all assays except for NK activity and NK cell enumeration, the positive control cyclophosphamide was administered by intraperitoneal injection (50 mg/kg) on Days 24-27 at a dose volume of 5 mL/kg. For NK cell activity and NK cell enumeration, the positive control, Anti-asialo GM1, was administered by intravenous injection (1 mL/animal of a 1:10 dilution) approximately 24 hours prior to scheduled necropsy.

For systemic toxicity, there were no treatment-related effects on body weights, body weight gains, food consumption, spleen weight, thymus weight, or gross pathology.

At 100 mg/kg/day, treatment-related clinical signs were noted in all treated animals 30-minutes after dose administration. These findings consisted of rocking, lurching, or swaying while walking; drooping eyelids; and subdued appearance upon cage-side observation. Clear material around the mouth and yellow material on various body surfaces (anogenital area, urogenital area, ventral trunk, hindlimbs, and/or around the mouth) were also observed in the animals at 100 mg/kg/day. Additionally, flattened body was noted in 8 rats, and 2 rats had hunched posture at this dose.

At 30 mg/kg/day, subdued appearance was observed in 5 rats in the AFC group for a total of 6 occurrences and in 4 rats in the non-AFC group for a total of 18 occurrences. Although considered treatment-related, in the absence of other clinical signs of toxicity, this finding was not considered adverse at this dose.

The only findings noted at the time of dosing or during the daily examinations that were considered treatment-related were oral secretions and urogenital/anogenital staining.

Positive control, cyclophosphamide, treated animals showed a few incidence of yellow material on various body surfaces (anogenital area, urogential area, ventral trunk, and hindlimbs). Significant ($p<0.01$) mean body weight losses were observed for overall (days 0-28) and days 24-28 in the cyclophosphamide treated animals compared to the negative controls.

For systemic toxicity, the LOAEL is 100 mg/kg/day based on clinical signs of toxicity (rocking lurching, or swaying while walking; drooping eyelids; subdued appearance at cage-side observation; flattened body; hunched posture; clear material around mouth; and yellow material on various body surfaces). The NOAEL for systemic toxicity is 30 mg/kg/day.

For immunotoxicity, there were no significant differences among treated and control groups in thymus and spleen weights and total spleen cell numbers. There were no significant treatment-related effects on antibody forming cell response (humoral immune response) to the T-dependent antigen (SRBC) and on the NK cell activity (innate immunity). Splenocyte surface marker differential analyses indicated that the absolute numbers of B cells, total T cells, T helper cells, T cytotoxic cells, natural killer cells, and monocytes/macrophages in the treated groups were comparable to negative controls. There were no significant differences among treated and control groups in the cell-mediated typed proliferative response in spleen cell cultures with or without the anti-CD3 antibody stimulation.

Positive controls, cyclophosphamide for antibody forming cell assay and Anti-asialo GM1 for NK cell activity assays, were adequately preformed to demonstrate the sensitivity of the assays.

Under conditions of this study, no immunotoxicity was observed. The NOAEL is 100 mg/kg/day (the highest dose tested).

This immunotoxicity study is classified acceptable/guideline and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800).

Appendix B. Physical/Chemical Properties

Table B.1 Physicochemical Properties of Demiditraz

Parameter	Value	Reference
Melting point/range	138.36 °C (range 137 – 142 °C)	MRID No. 47744103 B. Kitchens. 8/31/10
pH (23 °C)	Not applicable as technical grade demiditraz is not soluble in water.	
Density	0.15 g/cc	
Water solubility (mg/ml)	< 0.1 mg/ml	
Solvent solubility (mg/ml)	Acetone: 33 to 100 Acetonitrile: 33 to 100 Dichloromethane: 100 to 1,000 Dimethyl sulfoxide: 100 to 1,000 Ethanol: 100 to 1,000 Ethyl acetate: 10 to 33 Toluene: 1 to 10	
Vapor pressure	Not applicable as technical grade demiditraz is solid at room temperature	
Dissociation constant (pK_a)	Not applicable as technical grade demiditraz is a weak base and insoluble in water	
Octanol/water partition coefficient	The mean $\log_{10}P$ value reported is 2.78	
UV/visible absorption spectrum methanol (nm)	Shows maximum absorption at about 264 nm	